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SPOROGENESIS IN PALLAVICINIA.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXV.

ANDREW C. MOORE.

(WITH PLATES III AND IV)

THE presence of a quadripolar spindle in the division of the spore mother cell of *Pallavicinia decipiens* was announced by FARMER in 1893, and in 1894 he published his detailed studies upon the same plant. The most remarkable feature of these papers is the significance which the author attributes to a quadripolar spindle as a means for the simultaneous distribution of the chromatin to the four daughter cells which become the spores.

According to FARMER'S account (**5, 6**), the structure in question is developed quite early, before any evidence of approaching division is visible in the nucleus. Later the nucleus becomes lobed, and finally four chromosomes make their appearance. The number is increased by division to eight, which point off in pairs to the four lobes of the spore mother cell. "A further doubling of the chromosomes occurs, so that four of these bodies . . . . go to form the nucleus in each spore. The whole process is very much crowded up, the four-rayed spindle persisting to the end; and even after the exodus of the chromosomes, traces of it can still be seen converging to the original center."

The presence of a quadripolar spindle is of itself not surprising, since tripolar, quadripolar, and multipolar spindles have been frequently described by various authors; but in every case these structures represent early stages in the development of the achromatic figure and later become normal bipolar spindles. The peculiar

interest attaching to the structure described by FARMER is the reported distribution of the chromatin simultaneously to the four daughter nuclei. If his observations and his interpretation of the spindle are correct, Pallavicinia occupies a unique position among plants and animals.

FARMER (**7**) sought through a study of other liverworts to throw further light on this subject. He found the quadripolar spindle present in *Aneura pinguis*, *A. multifida*, *Scapania undulata*, Fossombronia, and in other types of the Jungermanniales, but in no case did he find it persisting and functioning, as in Pallavicinia, in the simultaneous distribution of the chromatin. In these forms, according to his interpretation, the ends of the quadripolar spindle fuse in pairs and the distribution of chromatin takes place in the usual manner through two successive mitoses. While not directly confirming his results on Pallavicinia, FARMER thinks the conditions found in these plants strengthen his position. He regards them as representing transitional stages between the normal type of division and the very unusual type which he reports in Pallavicinia. CAMPBELL (**1**) and other authors generally have accepted FARMER'S account.

DAVIS (**4**) from an investigation of Pellia was led to question FARMER'S conclusions. He regards the quadripolar spindle as a condition of prophase, and believes that it is always followed by two successive mitoses after the usual manner in the spore mother cell, each with a normal bipolar spindle. FARMER (**8**) is not willing to exclude the four-rayed figure from the spindle apparatus or to employ the term spindle in the restricted sense of DAVIS; but the main features of the discussion are not the questions as to when the achromatic structure becomes a spindle and as to the limitation of this term as a matter of usage—points upon which authors may readily disagree. The fundamental differences between the views of DAVIS and FARMER lie in the history of the quadripolar spindle, and the method by which the chromatin in the spore mother cell is distributed to the four spores. FARMER positively asserts that the quadripolar spindle retains its form and that the chromatin is distributed simultaneously to the four daughter nuclei. DAVIS believes that the quadripolar spindle is a condition of prophase which is followed by two successive

mitoses, each with bipolar spindles, by which the chromatin is distributed in the usual way within the spore mother cell. Apart from the rapidity of the two mitoses and the prominence of a four-rayed achromatic figure in the prophase of the first, the latter author holds that there is no essential difference between the processes of sporogenesis in Pallavicinia and in other liverworts and higher plants. DAVIS (**4a**) further maintains these opinions in his recent review of the events of nuclear division within the spore mother cell.

In view of the unusual character of FARMER'S results and of the fact that doubt has been expressed as to the accuracy of his observations and their interpretation, I have undertaken an investigation of *Pallavicinia Lyellii*, believing that evidence obtained from the study of another species of the same genus would help in clearing up the disputed points. Some of my results (**15**) have already been published, and they do not confirm FARMER in his main contention, namely, the simultaneous distribution of the chromatin.

Pallavicinia Lyellii is a cosmopolitan species which I have found growing abundantly near Columbia, S. C., and in the vicinity of Woods Holl, Mass. The young sporophytes make their appearance in the early fall and mature about the first of April. The material was fixed in chromo-acetic acid and stained with saffranin and gentian violet alone, or in the triple combination of saffranin, gentian violet, and orange G. Iron-alum haematoxylin was also used after the method of Haidenhain. Upon the whole the last-named stain has given the best results. The fibrillar structures are not so well brought out by it as by the gentian violet, but the chromosomes are much more clearly differentiated.

The spherical resting nucleus occupies a central position in the distinctly four lobed spore mother cell. It enlarges considerably preparatory to division and becomes somewhat angular, extending into the lobes of the spore mother cell. At the period of synapsis the nucleolus is conspicuous for its size and prominence (*fig.* 1), as is also the confused tangle of chromatic threads. The spore mother cell is not so deeply lobed as FARMER (**8**) figures for *Pallavicinia decipiens*, and DAVIS (**4**) and CHAMBERLAIN (**2**) for Pellia.

FARMER did not observe the spirem of *Pallavicinia decipiens*. In *P. Lyellii* it is exceedingly well-developed, and immediately after

synapsis is observed as a very definite linin thread in which deeply staining chromatic droplets (*fig. 2*) occur at intervals. This spirem is loosely wound in many convolutions through the nuclear area and shows no signs of fine anastomosing filaments uniting its parts. The nucleolus is not so conspicuous as in the preceding stages of synapsis.

The spirem thread shortens and thickens and the chromatin granules become larger and less numerous (*fig. 3*). This process continues until the length of the whole thread is not more than that of the circumference of the nucleus, though it does not always occupy a peripheral position. During the latter part of this shortening process, there is a crumpling of the thread and a crowding together of its chromatin granules. This condition is of comparatively short duration and it is at this point that I observed the first evidence of a double thread (*figs. 4* and *5*).

The thread presently segments into eight chromosomes which lie scattered about in the nucleus in the form of a ring. These eight chromosomes are irregular in shape and frequently show with great clearness that they are not homogeneous masses, but made up of individual parts. I was at first in much doubt as to the number of parts, but subsequent study has convinced me that there are four, and that we are dealing here with tetrads. FARMER (**7**) shows very clearly by his figures of Fossombronia that he saw a similar arrangement. He says "sometimes four such aggregations could be seen in each chromosome, but the number was not sufficiently constant to afford very secure ground for theorizing." However, he expresses the opinion that we have here a double longitudinal split, in which the chromosomes are already prepared for the two succeeding divisions.

The tetrads are clearly shown in *fig. 8*. The appearance of several of the eight chromatic masses suggests very strongly that they are made up of four parts, while the evidence presented by the one in the center is conclusive. Here there is present the ring form, and the four elements of the tetrad are quite distinct. Several of the forms assumed by the tetrads are illustrated in *fig. 10*, viz., crosses, Ys, Ts, and rings. The fourfold nature of the group is most clear in the ring form. In *fig. 10 a* the four daughter chromosomes of the

tetrad are shown entirely separated from one another. *Fig. 11* illustrates another case where the daughter chromosomes of the tetrad are separated. In this figure the two groups are drawn in correct relative positions, the one showing an almost homogenous mass, the other four daughter chromosomes. The fourfold character of the chromatic masses is most evident immediately after the segmentation of the chromosomes. Very soon they become more compact, and while they continue to show irregularities in outline, up to the metaphase of mitosis, they are not so evidently composed of four elements. *Fig. 9* presents a stage somewhat later than *fig. 8*.

I have not been able to determine the origin of the tetrads with any certainty. *Fig. 6* would seem to indicate that the elements of the tetrads are formed previous to the segmentation of the spirem and that these in some way become properly grouped. The large number of chromatic elements, together with the differences in their size and shape in this figure, are no doubt to be correlated with the different degrees to which the aggregations have progressed in the formation of the tetrads. In *fig. 7* the number of masses has been reduced, their size is approximately uniform, and the time has almost arrived for the segmentation of the chromosomes. Until the origin of these tetrads can be made out definitely, it would be useless to theorize concerning them.

While the foregoing changes take place within the nucleus, the outer form of that body is altered. It becomes strongly lobed, often assuming a tetrahedral form, one angle projecting into each lobe of the spore mother cell. FARMER (**7**) describes a similar form in several of the Jungermanniales studied by him, and attributes it to a pull by the four centrosomes which he finds in the four lobes of the mother cell. In describing the process for Fossombronia he says "the nuclear wall is not broken, although it becomes greatly pulled out beneath each centrosphere, and thus the quadripolar spindle is so far a nuclear distortion."

While the tetrahedral form is perhaps the most usual at this stage, it is by no means the only one. Frequently there are more than four projections. Such a condition as is illustrated in *fig. 12* would require the assumption of more than four centrosomes. In many cases the lobes of the nucleus are rounded and do not indicate

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that they are caused by a pull upon the nuclear membrane (*fig. 3*). Besides, the membrane in sections can be seen to be wavy, showing that it is not under tension from a dynamic center. The lobing occurs long before fibrillar elements are visible, and if the assumption that fibers are the expression of lines of force be true, then such lines of force do not exist at this time, and hence the irregularities in the shape of the nucleus cannot be attributed to a pull by them. It would seem much more probable that they are due to an amoeboid movement of the nucleus. It is well known that the nucleus of certain cells possesses this power, and observers have noted the phenomenon in living cells. It has also been noted that there is in a measure a correspondence between the shape of the nucleus and that of the cell to which it belongs. When the cell is much attenuated the nucleus is greatly elongated. In *fig. 28* is shown a resting nucleus from an elater of Pallavicinia. Miss MERRIMAN (**14**) discusses this question in relation to the differentiation of tissues from the meristem in the root tips of Allium and attributes to the nucleus the power of amoeboid motion. KORSCHELT (**12**) describes in the egg of the water beetle Dytiscus a nucleus with pseudopodia-like processes extending out into a mass of granular food particles.

As previously stated, the resting nucleus of the already deeply lobed spore mother cell is spherical. In preparation for division the great changes which take place in its size and in the character of its contents must be connected with great metabolic changes going on within it. The materials necessary for the supply of this demand must come from the cytoplasm, which in this case consists of four masses occupying the four lobes of the spore mother cell, and the reaching out of the nucleus for food might tend to produce a tetrahedral form.

According to FARMER the quadripolar spindle was the first evidence of approaching division. He says the first evidence noted in the nucleus itself was the collection of four chromatic droplets in the center at a time subsequent to the appearance of the four-rayed structure. In *Pallavicinia Lyellii*, as has already been pointed out, the changes which take place in the nucleus itself indicate approaching division before any structure makes its appearance which could be interpreted as a quadripolar spindle.

FARMER does not discuss the origin of the achromatic spindle, evidently regarding that as a matter of minor importance as compared with its later behavior in his account of the simultaneous distribution of the chromatin. The study of the origin and development of the achromatic structure of *Pallavicinia Lyellii* is attended with considerable difficulties, owing to the large number of chloroplasts in the cell. However, it seems to conform in general to the type described by DAVIS (**4**) for the corresponding phase of Pellia. He finds that kinoplasmic caps form over the lobes of the nucleus and extend down over it, finally forming fibrillae which enter the nuclear area. In my preliminary note (**15**) I described a similar process for Pallavicinia. I found aggregations of kinoplasm at the angles of the nucleus, and out of this material fibers are formed, which extend down over the protruding portion of the nucleus. FARMER (**8**) has recognized in one of my figures representing this stage the same structure as his quadripolar spindle.

In *P. Lyellii* this structure is never so prominent as that described by FARMER, but his figures do not distinguish clearly the spindle fibers from the nucleus. My preparations show a decided lobing of the nucleus, but with very slight indications of differentiated fibrillar protoplasm over the lobes. I find no astral rays and no evidence whatever of the existence of centrospheres or centrosomes. DAVIS (**4**), CHAMBERLAIN (**2**), and GRÉGOIRE and WYGAERTS (**9**) find asters and kinoplasmic caps well developed in other periods of ontogeny, but do not find them so prominent, if at all, in the spore mother cell. FARMER indeed does not mention the presence of asters in Pallavicinia, nor does he figure them. DAVIS (**3**) in his investigation of Anthoceros was the first to question the presence of centrosomes in the spore mother cell of liverworts. My studies lead me to hold similar doubts and to believe with him that the spindle fibers in the spore mother cells of liverworts develop independently of centrosomes, so that multipolar stages in spindle formation may be expected, as OSTERHOUT, MOTTIER, and JUEL established in 1897 in the pteridophytes and spermatophytes.

CHAMBERLAIN (**2**), who studied the germinating spore of Pellia with special reference to the centrosome problem, describes a peculiar structure in the form of a vesicle fitting over the end of the nucleus,

and in this he is confirmed by GRÉGOIRE and WYGAERTS (**9**). This vesicle, which he interprets as a *Hautschicht*, resolves itself into fibers and furnishes at least a part of the material for the spindle. I do not find such a vesicle separate and distinct from the nuclear membrane, but I find strong evidence that the nuclear membrane itself becomes resolved into fibers. This view is quite compatible with the generally accepted theory of the nature of a plasma membrane, and the evidence is presented by such appearances as are shown in *figs. 12–14*. In *fig. 12* we have a nucleus which in one plane shows a number of prominent lobes. A few fibers are visible over one lobe, and at several other places the nuclear cavity is apparently bounded by a weft of fibers. These are either derived from a layer of kinoplasm which closely invests the nucleus or from the nuclear membrane itself. The fact that the nuclear membrane disappears as these fibers come into view would lend force to the latter supposition. In *fig. 13* fibers are shown over one lobe of a nucleus which is very much elongated, and in *fig. 14* they may be seen at both ends of a similarly elongated nucleus. In the latter case the nuclear membrane persists in several places, seeming to merge gradually into the fibrillar condition. The fibers appear to conform to the irregularities of the surface, giving strong indications that they are derived from the nuclear membrane.

HARPER (**10**) has shown a close relation between membranes and fibers in Erysiphe, where, in free spore formation in the ascus, the fibers which mark out the boundary of the future spore fuse side by side to form a plasma membrane. The nuclear membrane is generally believed to be of kinoplasmic origin, and so are the fibers of the achromatic spindle. Evidently then, the transition from the one to the other may be easily accomplished.

Soon after the appearance of the first fibers, the number is greatly increased, but I have not been able to determine the origin of the remainder. The completed spindle is bipolar, and may be pointed (*fig. 15*) or blunt (*fig. 16*). The ends may terminate near or at a distance from the cell wall. It happens frequently that one end extends into a lobe of the spore mother cell, and the other abuts on the infolded wall between the two adjacent lobes which stand opposed to it, thus producing a very much flattened pole or even a forked one (*fig. 16*).

At the completion of the achromatic spindle, the chromosomes are found grouped in a ring at the equatorial region of the structure. *Figs. 8* and *9* show the arrangement of the chromosomes at this stage. *Fig. 15* gives a side view, slightly oblique, of the chromosomes at metaphase of mitosis. Five chromosomes are in view and the other three are hidden or have been removed by the razor in making the section.

I have not been able to make out satisfactorily the details of the separation of the daughter chromosomes. The distribution is effected very quickly, for great numbers of nuclei in metaphase have been observed and a great many in telophase, but very few in anaphase. Little indication is given as to the exact manner in which the separation takes place. A few instances of chromosomes as they are pulled apart are shown in *fig. 17*. The appearance of the chromosomes indicates beyond doubt that they are plastic bodies subjected to a pull, and that they are being halved; but what real relation this distribution bears to the original tetrads is left in doubt. In *fig. 18* we have shown anaphase in which the chromosomes are somewhat scattered upon a very broad spindle. There are five near each pole and one almost half way between. It is evident that the remaining chromosomes are upon another section.

During telophase the chromosomes are found arranged in compact rings at the two poles. When one end of a spindle abuts on a dividing wall between two lobes, the ring at that end sometimes lies very close to this wall, partially surrounding it (*fig. 19*).

There is no resting stage between the first and second mitoses. The chromatic elements of the nucleus do not resolve themselves into a reticulum and the chromosomes do not lose their individuality. The rings of chromosomes which have been formed at the telophase of the first division merely alter their positions, so that their planes lie at right angles to one another. It is evident from *fig. 20* that the chromosomes come in contact and form a thick spirem, but do not lose their identity. This is the nearest approach to a resting stage I have been able to find, and I believe it is unusual for the reconstruction of the nucleus to proceed even this far. No nuclear membrane is formed at the end of the first mitosis and no cell plate is laid down. In a few instances granules were seen across the equatorial portion

of the spindle, but the process of forming a wall seems to go no further; indeed, it very seldom proceeds to this point.

That the second mitosis succeeds the first very closely is attested by the fact that examples of both divisions are frequently found in the same capsule. The spore mother cells of a given capsule are in division at the same time, though not exactly in the same phase of mitosis. Occasionally cells are found which lag considerably behind or precede the majority in division. Such cases are of great value in determining stages with certainty.

The spindles for the second mitosis make their appearance very suddenly, and I have not been able to determine their origin. They are quite strongly developed, and as a rule are longer and narrower than the spindles of the first mitosis. The passage from the metaphase to the telophase is almost as rapid as in the first division, and no additional evidence is afforded as to the manner in which the chromosomes separate. *Fig. 21* illustrates metaphase of the two spindles, showing a polar and a side view. In this example the poles of the spindles are sharply pointed. In *fig. 22*, which represents an anaphase, the poles are blunt. The chromosomes pass rapidly to the poles and are grouped at the two ends in rings (*figs. 23* and *24*). At this stage the fibers are very prominent in transverse sections of the spindle (*fig. 24*).

Soon after the chromosomes have passed to the poles, granules make their appearance upon the equatorial region of the spindle (*fig. 24*). These become divided and a cell plate is laid down between them (*fig. 25*). Meanwhile the nuclear membrane is formed and the chromatic elements pass over into the reticulum characteristic of the resting state.

Finally, the new cell plates unite with the folded walls between the lobes and the separation of the spores is complete (*fig. 26*). The contiguous walls split apart and the spores become free. They next increase in size, becoming almost spherical, and the wall thickens and is finally marked with delicate points (*fig. 27*).

The spores do not germinate in the capsule as do the spores of Pellia. Soon after being shed, they increase greatly in size, stretching the wall, as is clearly shown by the separation of the points upon its surface. After the cell has attained a size several times that of the

original spore, the first mitosis of the gametophyte generation takes place.

I have not been able to contribute much to a knowledge of the behavior of the nucleolus. It stains like the chromosomes most of the time, and when the latter are differentiated it becomes difficult to identify the nucleolus with certainty. During synapsis the nucleolus is a very large and conspicuous body (*fig. 1*). It is not so large during the later spirem stages, but still quite prominent (*figs. 2* and *3*). At the time the spirem is ready to segment, the nucleolus shows a slight difference in staining reaction from the chromosomes. With the saffranin and gentian violet combination it takes slightly more gentian violet, and with the iron-alum haematoxylin it stains less intensely than the chromosomes. At this time it shows signs of fragmentation (*figs. 6* and *9*).

Various theories regarding the constitution of the nucleolus have been advanced: one that it is achromatic and contributes to the formation of the spindle; another that it is chromatic and contributes to the formation of the chromosomes. WAGER (**18**) in a recent paper attributes to it important functions in the organization of the chromosomes and in the transmission of the hereditary substance. Its staining reactions would seem to ally it more closely with the chromatic elements of the cell. If the nucleolus plays a part in the formation of the achromatic spindle in the first division of Pallavicinia, it certainly does not in the second, since there is no reconstruction of the nucleus and the nucleolus is not reformed. Upon the whole the evidence, though by no means conclusive, indicates that the nucleolus in Pallavicinia may be regarded as contributing to the chromatin.

FARMER (**6**) states that there are four chromosomes in *Pallavicinia decipiens*. In *P. Lyellii* I find eight as the reduced number in the spore. The count is very easily made when a polar view is obtained, and the compact form of the chromosomes makes the task an easy one. The chromosomes are in most favorable position for counting when viewed from the poles during metaphase and early telophase, as the figures clearly show (*figs. 8, 9, 21, 23*).

In *fig. 23* it will be observed that there are nine chromosomes in one group. It is possible that the sister group would show only seven. In the same figure, upon the conspicuous spindle which is

cut longitudinally it is uncertain to which group the chromosome lying near the middle belongs. *Fig. 18* shows that the chromosomes do not always pass simultaneously to the poles, and it is possible that the distribution is not always equal. I have frequently been able to count only seven chromosomes in a group, but such evidence is uncertain, since there is always the possibility that one has been removed by the razor in making the section. In case the number exceeds eight the difficulties are fully as great, since there is always the possibility of a tetrad being broken apart. It is true that in such an example the size of the bodies is some check, but still there is great uncertainty. Also the nucleolus, which as has been stated stains as the chromosomes, is to be reckoned with, if the count is made at a stage when that body is present. However, I believe that while the number of the chromosomes is normally eight, occasionally a variation from this number will be met, due no doubt to an unequal distribution during division.

The number of chromosomes in the sporophyte is undoubtedly sixteen, though I have not made an actual count. *Figs. 29* and *30* represent the two parts into which a single cell of the seta has been cut. It will be observed that the spirem is just segmenting into the elongated chromosomes; two nucleoli are still visible (*fig. 29*). The count cannot be made with absolute certainty, but the number is approximately sixteen. *Fig. 31* shows one section of an early telophase from a cell of the seta. There are seven and eight chromosomes at the respective poles. The other section of the same cell shows about the same number of chromosomes in each group, but the masses are too confused to admit of an accurate count. I have observed figures in dividing spermatogenous cells, and here also the number of chromosomes is without doubt sixteen.

It seems desirable to point out that my final conclusions agree in all essentials with my preliminary paper of 1903, and are in conflict with FARMER'S views in the fundamental feature of his account of Pallavicinia—the simultaneous distribution of the chromatin to the four daughter nuclei through a quadripolar spindle. It is perfectly clear from my studies that the chromosomes in *Pallavicinia Lyellii* are distributed by two successive mitoses, each with well-defined bipolar spindles, and that the chromosomes are organized

as tetrads just before the first mitosis. The achromatic structure which corresponds to FARMER'S quadripolar spindle appears during the prophase of the first nuclear division, and is followed by clearly defined bipolar spindles of the two successive mitoses with no evidence of accompanying centrosomes. The events of sporogenesis in *Pallavicinia Lyellii* present then no fundamental differences from those of other liverworts and higher plants, the chief peculiarity being the rapidity with which the second mitosis follows the first.

SUMMARY.

1. The resting nucleus is spherical in shape and centrally situated in the spore mother cell. The spore mother cell is deeply four-lobed at an early period in its history.

2. During synapsis the nucleus, containing a large and conspicuous nucleolus and a contracted chromatic thread, enlarges and becomes irregularly lobed.

3. There is a distinct spirem stage in which a clear cut linin thread bears deeply staining chromatin granules. The thread shortens and thickens and at the same time the granules become larger and less numerous.

4. The first evidence of a double spirem is observed just previous to the segmentation of the thread.

5. The spirem segments into eight tetrads, which may be in the form of rings, Xs, Ys, Ts, or irregular masses.

6. While these changes are taking place within the nucleus, the membrane becomes strongly lobed. Frequently, though not always, the form of the nucleus is tetrahedral, the angles projecting into the respective lobes of the spore mother cell.

7. There is no direct evidence of centrosomes or centrospheres and the indirect evidence is against their presence.

8. The lobing of the nucleus is due to amoeboid motion in response to nutritive stimuli.

9. The achromatic spindle originates in kinoplasmic caps to which the nuclear membrane contributes material.

10. The distribution of the chromatin is effected through bipolar spindles in two successive mitoses.

11. There is no resting stage between the first and second mitoses.

12. The two bipolar spindles of the second mitosis are strongly developed and stand at right angles to each other.

13. After the second mitosis, cell plates are formed and the nuclei pass into a condition of rest in the usual manner.

14. The spores do not germinate in the capsule before its rupture, as do those of Pellia.

15. The nucleolus is more closely allied to the chromatic than to the achromatic material of the nucleus.

16. The number of chromosomes for the gametophyte is eight and for the sporophyte sixteen.

This investigation was conducted under the direction of Professor BRADLEY M. DAVIS, to whom I desire to acknowledge my indebtedness for valuable criticism and suggestion; as well as to Professor JOHN M. COULTER and Dr. CHARLES J. CHAMBERLAIN.

SOUTH CAROLINA COLLEGE,
Columbia, S. C.

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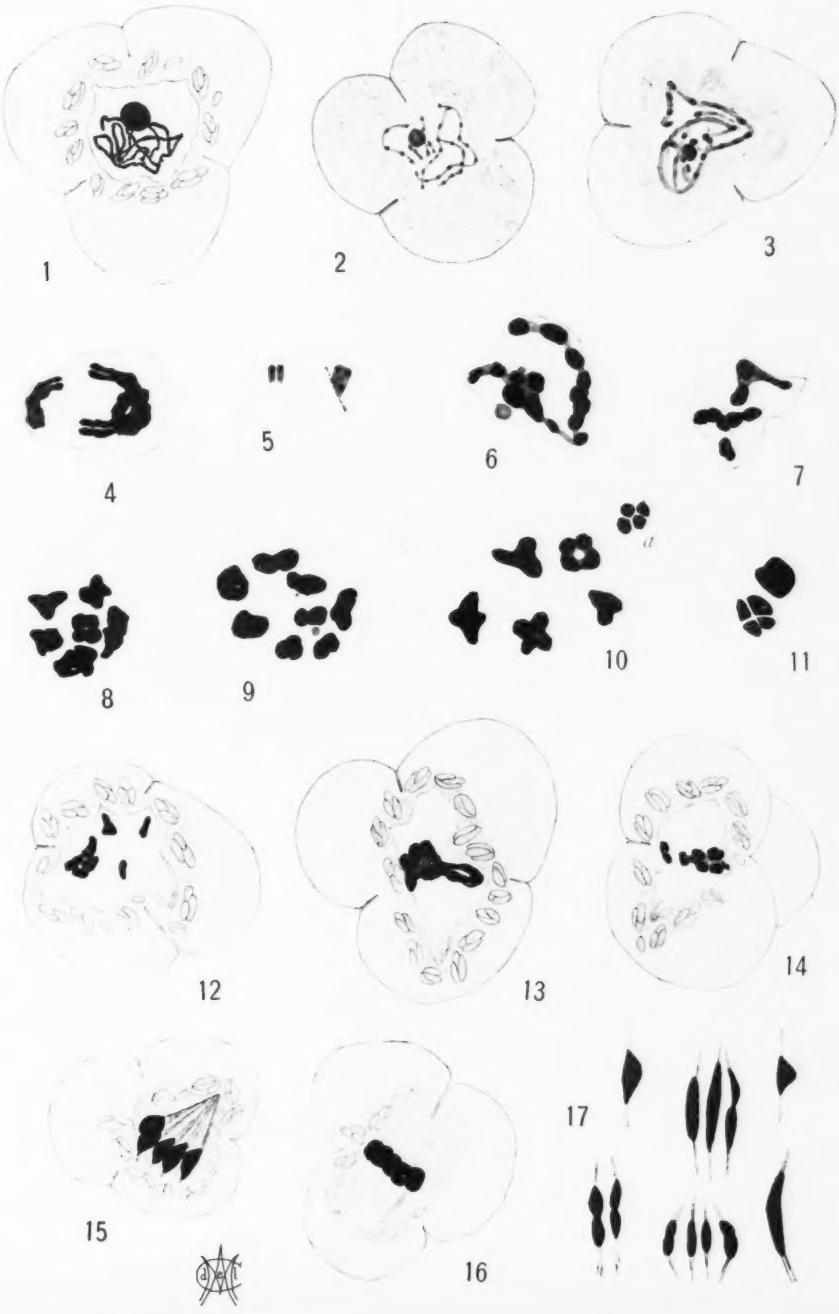
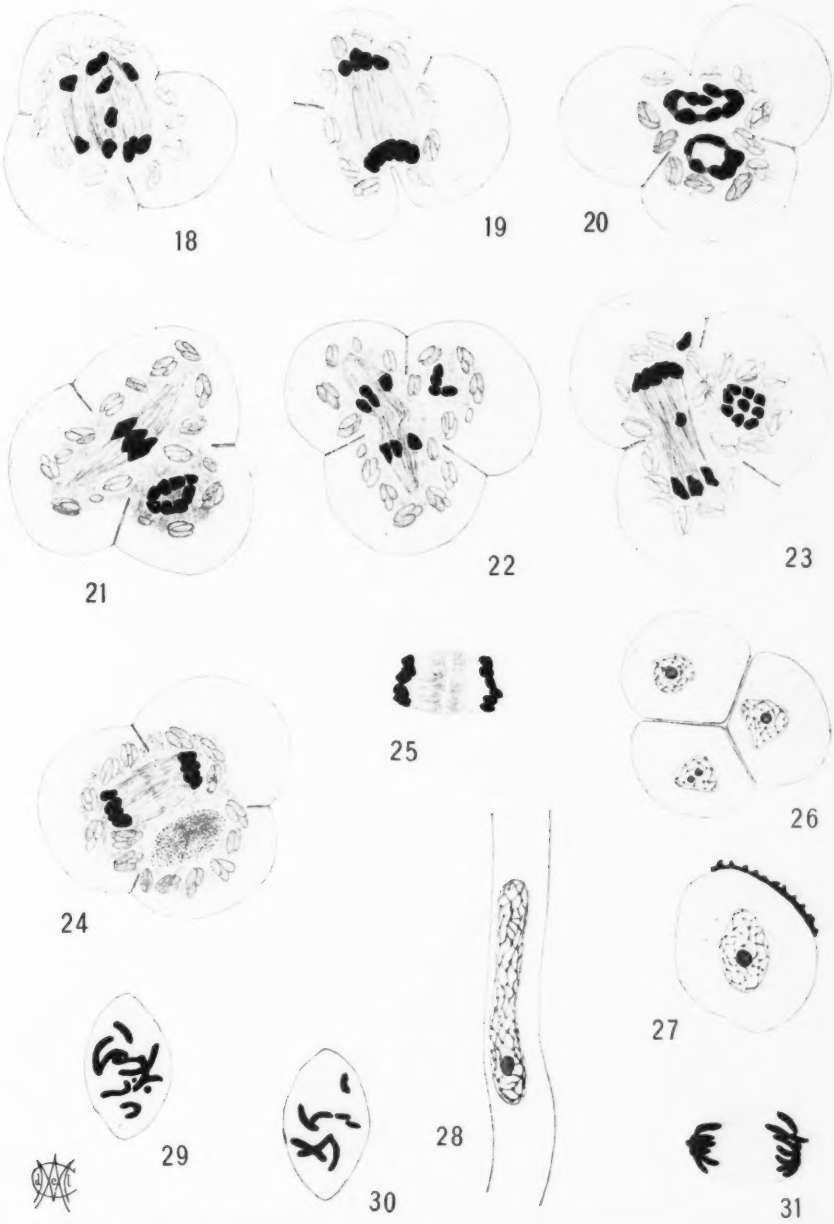


MOORE on PALLAVICINIA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EXPLANATION OF PLATES III AND IV

All figures except *fig. 28* were made with a Zeiss 2^{mm} apochromatic objective and a no. 12 ocular. *Fig. 28* was made with the same objective, but with no. 8 ocular. A Bausch and Lomb camera lucida was used for all drawings. In *fig. 2* all the plastids of the cell are shown; in other cases only those immediately surrounding the nucleus.

FIG. 1. Enlarged nucleus of spore mother cell in early stage of preparation for division; the nucleolus is conspicuous and the appearance and arrangement of the chromatin indicate the condition of synapsis.

FIG. 2. Spirem condition, showing linin thread loosely wound with deeply staining chromatic droplets at intervals.

FIG. 3. Thicker and shorter spirem; chromatic droplets fewer and larger; lobes of nucleus distinctly rounded.

FIG. 4. Spirem further shortened; chromatic droplets crowded together; the thread appears double.

FIG. 5. Ends of spirem thread, showing that it is double.

FIG. 6. Aggregation of chromatic droplets just previous to segmentation of chromosomes; probably time of tetrad formation; the nucleolus seems to be fragmenting.

FIG. 7. Later stage than *fig. 6.*

FIG. 8. Equatorial plate stage, showing group of eight tetrads.

FIG. 9. Equatorial plate stage, later than *fig. 8;* tetrads not so clearly defined; nucleolus fragmenting.

FIG. 10. A group of selected tetrads, showing rings, crosses, Ys, and Is; *a*, tetrad resolved into its elements.

FIG. 11. Neighboring tetrads of an equatorial plate; in one the fourfold character is clear, while in the other it is obscured.

FIG. 12. Prophase of first division; nucleus many-lobed; fibers over the largest lobe and at other places on the surface of the nucleus.

FIG. 13. Spindle organizing for first division; spindle fibers prominent on one end, approaching bipolar condition.

FIG. 14. Bipolar spindle of first division; nuclear membrane resolving into spindle fibers.

FIG. 15. Oblique side view, metaphase of first division; end of spindle pointed.

FIG. 16. Metaphase of first division, showing one very flat and one forked pole.

FIG. 17. Dividing chromosomes.

FIG. 18. Anaphase of first division, showing chromosomes scattered.

FIG. 19. Telophase of first division, showing grouping of chromosomes in rings at the poles.

FIG. 20. Beginning of reconstruction of daughter nuclei at completion of first division; the chromosomes do not lose their identity and no nuclear membrane is formed.

FIG. 21. Metaphase of second division, showing side view of one spindle and polar view of the other; in the side view the poles are seen to be pointed and in the polar view eight chromosomes appear.

FIG. 22. Anaphase of second division, showing blunt poles.

FIG. 23. Telophase of second division, showing nine chromosomes in the polar view of one of the spindles.

FIG. 24. Telophase of second division, showing beginning of cell plate in one spindle and transverse section of fibers in the other.

FIG. 25. Formation of cell plate.

FIG. 26. Completed spores with resting nuclei and separating walls.

FIG. 27. A single spore which has increased in size and has attained its thickened and roughened wall.

FIG. 28. Resting nucleus of elater.

FIG. 29. Segmenting spirem of cell from seta of sporophyte, showing nine chromosomes.

FIG. 30. Remainder of the same cell, showing seven additional chromosomes, making sixteen in all; chromosomes differ in shape from those of the dividing spore mother cell.

FIG. 31. Early telophase of cell from seta; only half of the cell is shown; there are eight chromosomes at one end and seven at the other; the neighboring section makes it evident that the total number is sixteen at each end.

REGENERATION IN PLANTS. I.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXVI.

WILLIAM BURNETT MCCALLUM.

(WITH FOURTEEN FIGURES)

INTRODUCTION.

THE term regeneration has come to be used by most botanical writers with a broad and somewhat indefinite application. Its essential feature, however, is the replacement of an organ or structure that has been removed. This is accomplished in a variety of ways. PRANTL (**9**) first found and later SIMONS (**11**) determined more accurately that if the tip of a root be cut off not more than 0.75^{mm} from the end there is a complete restoration of the part removed, a new tip forming out of the tissues at the cut surface. GOEBEL (**3**, p. 503) has shown that if the young apex of the frond of Polypodium be cut in two lengthwise, the remaining embryonic tissue on each piece will completely reform the half that has been removed. The same is true of the growing point of a fern prothallium, although the older parts are not replaced. These phenomena are quite homologous with regeneration as it occurs in animals. If we cut off the root tip somewhat farther back, however, a new tip is not organized at the cut surface, but behind it one or perhaps more new root primordia are organized, and these take the place of the main root. Or if we cut off transversely a portion of the thallus of Marchantia or Lunularia (**12**), the tissues at the cut surface will not develop, but there will arise from apparently mature and differentiated cells back of the cut new outgrowths of thallus which again will complete the plant.

If the shoot with all the buds be severed from the root of Taraxacum, new shoots will arise lower down from the mature tissues of the cortex. Many fleshy roots have this capacity, and if cut into a number of pieces each will organize new primordia and develop shoots. If the young stem of Convolvulus, Linaria, and other plants (**6**) be cut off just below the cotyledons, there will arise on the sur-

face of the hypocotyl outgrowths which develop into new shoots. These shoots also arise from mature cells which in the normal course of events remain as permanent tissue. Nor is this power of organizing new shoot primordia confined to the stems and roots, but is also possessed by many leaves, as in the well known cases of Begonia, Bryophyllum, *Cardamine pratensis*, *Tolmiea Menziesii*, and many other plants (*fig.* 1). Many stems, probably the majority, if removed from the root system and kept moist will produce new roots. In a few cases, as in Salix, there may exist on the stem primordia already organized, but in the great majority of stems these are not present. If a portion of the stem of Salix be cut out from the rest and kept moist, there will appear on it both roots and shoots, each arising, however, from buds already laid down. In the axils of the leaves of many annual shoots are very minute bud primordia, which normally do not develop. If the top of the plant be cut off, these at once form new shoots. In our trees and shrubs the buds formed in the leaf axils do not develop until the following year; but if at any time during the spring the tip of the young shoot be removed, a number of these buds, usually those near the top, at once develop shoots.

FIG. 1

We have in these cases at least three seemingly diverse phenomena: (1) the part removed is entirely restored by the growth of the cells immediately at the cut surface; (2) there is no growth of embryonic tissue at the wounded surface, but at a greater or less distance from it the organization of entirely new primordia which develop organs that replace those removed; (3) the organ removed, *e. g.*, the shoot, is restored by the development of already existing dormant buds. Between these no hard and fast lines can be drawn, for they all exhibit intergradations, and between the third case—the development of latent buds—and normal vegetative growth no sharp separation can

be made, for occasionally in some species, *e. g.*, Salix, the axillary buds on the first year's growth instead of remaining dormant until the following spring will develop at once into shoots.

It will be quite apparent that as regeneration merges so insensibly into ordinary vegetative growth, the necessary limitations as to the use of the term must be entirely artificial. PFEFFER (**8**) restricts the term to those cases where an organ directly replaces that portion of itself that has been removed; all others he would call mere reproduction. GOEBEL, KLEBS, MORGAN, KÜSTER, and most other writers on the subject, give it a broader meaning, so as to include the replacement of parts or organs, whether by means of entirely new growths, or from the development of latent buds. The advantage in having some general expression to cover all these phenomena, and the fact mentioned by MORGAN, that they all accomplish the same result and are probably due to the same cause, make it a matter of convenience to use the term in its wider application.

A certain amount of confusion has arisen because it has not been kept clear that regeneration is not really different from ordinary vegetative growth. Most plants naturally tend to grow and branch indefinitely, the new members arising usually in definite places, the shoot primordia, for example, in the embryonic parts of the shoot, and the root primordia ordinarily in the younger regions of the root. The fact that this is the general rule has led to an unjustifiably rigid limitation of the origin of new members to specified regions. As a matter of fact, the ability to produce new members is distributed throughout the plant body, and in many even of the higher plants almost any part is able to produce any other vegetative part. Nor is this ability limited to embryonic parts, for in very many plants it is exercised by the older cells, as in the production of shoots on roots of Taraxacum or on leaves of Begonia. That certain conditions are necessary to bring this latent ability into activity does not make it in the least different from ordinary vegetative growth, for the latter also is dependent on definite conditions.

The whole plant body of mosses and liverworts, and many roots, stems, and leaves of the vascular plants have this capacity, and it requires only the proper conditions to become manifest.

In spite of the extensive investigations into this question, ranging

as they have throughout the greater part of the plant kingdom, our knowledge of what these conditions are is very obscure. We know little enough of the external factors concerned and almost nothing at all definite about the internal ones. When a part of the plant body is removed, many factors are necessarily disturbed. The nutritive conditions may be profoundly altered, as also may be the water relation. The influence of the wound itself may be important, and independent of these the mere absence of the organ may in itself be of far reaching influence. How far any of these may be responsible for regeneration is not clear. Various theories have been proposed, but none have as yet been supported by adequate experimental evidence.

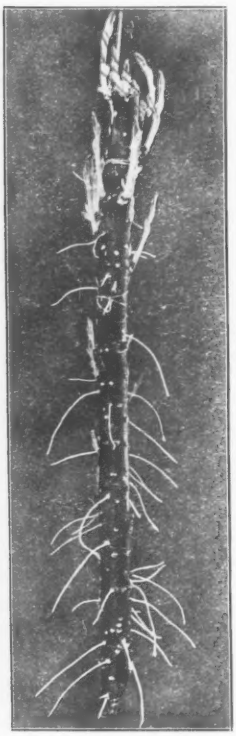

FIG. 2

Intimately associated with this problem of regeneration is that of polarity, for almost invariably the new structures occur in such a manner as to exhibit this remarkable phenomenon (*fig. 2*), and if we can determine the exact cause of the appearance of roots or shoots in an isolated piece of Salix stem, for example, the reason for their development at certain places only may be apparent. At present we are blocked at the outset by not knowing, at least under most circumstances, the stimulus which incites their development at all.

In conducting some investigations in this subject it soon became evident that the best method of attack would be to take all the possible factors and work on them separately, subjecting each, one at a time, to a more exact physiological analysis. The effort was made to determine whether the proposed cause in any given case of regeneration is a necessary part of the stimulus, by endeavoring to devise conditions under which the regeneration could be induced to occur in its absence. By this process of

exclusion the essential factor might be isolated. Experiments were conducted on Phaseolus, Salix, Helianthus, Taraxacum, Tolmiea, and other plants.

If the common scarlet runner bean, a variety of *Phaseolus multiflorus*, be cut off any place along the epicotyl, there arise from within the cotyledons two shoots, which grow vigorously and may attain the size of the normal plants (*fig. 3*). Sometimes, however, one of these may grow weakly, or even be entirely suppressed. These arise from two minute primordia which are present, one in the axil of each cotyledon. Of the many hundred plants under observation scarcely a case was seen in which these primordia developed without the removal of the shoot, and in every case in which the stem was cut off they developed. Though less than a millimeter in length, in three or four days after the stem is removed they appear above the cotyledons, and in a week are often 6^{cm} long. Growth is then very rapid, and in a month they may be 60 or 70^{cm} high. In the axils of each of the foliage leaves on the plant there is, as usual, a bud. These under the condition of my experiments rarely developed. If the plant be allowed to grow until the second internode is formed, and this be cut off, these dormant buds in the axils of the leaves at its base will at once become active and give rise to two shoots; or if the plant form several internodes and the upper one be removed, the buds of the nodes below (not necessarily the first one) at once will develop shoots.

FIG. 3

Here we have one of the most common phenomena in regeneration, namely the removal of a part stimulating to development what would otherwise be dormant primordia. But this same removal or isolation of a part is followed by the growth of organs where their primordia

do not exist; for if the stem of the bean be severed from the root system and kept moist, new roots appear along the stem. Similarly, when many roots and leaves or other parts are cut away, new shoot primordia are organized from cortical or other tissues, and it seems quite probable that the same stimulus which starts the development of many latent primordia will in many cases where they are absent incite their origin from already differentiated tissues.

What now are the possible factors operating in these cases? The various theories and possibilities suggested fall naturally into a few general classes: (1) wound stimulus; (2) disturbance in nutritive relations; (3) changes in water content; (4) accumulation at certain places of definite formative substances; (5) correlation; (6) relative age and degree of maturity of the different parts of a member; and (7) growth tensions. Each of these will be discussed in connection with the experiments relating to them.

Unless otherwise stated, the plant used was Phaseolus, and in every experiment a sufficient number of plants were used to insure reliability of the result, and check experiments were always carefully arranged. Where there was any diversity in the result the experiment was always repeated. Of the total number of experiments only the more striking ones will be described, and for convenience in the discussion these will be numbered in the order in which they are presented.

DISTURBANCE IN NUTRITION.

When a growing part is removed, a large part of the food that would have been used by it may now be unused in the plant, and may be accessible to other parts. If during the spring the tip of a shoot of almost any tree be removed, some of the buds which would otherwise lie dormant until the next year develop into shoots (*figs. 6* and *7*). Here, and in Onoclea where GOEBEL (**2**) secured the metamorphosis of sporophylls into foliage leaves by the continued removal of the former, KLEBS (**5**) sees an essential factor in the disturbance occurring in the nutritive conditions. In the axils of the cotyledons of *Juglans regia* there are a number of buds which, as GOEBEL (**4**, p. 209) has pointed out, do not develop unless the terminal bud be removed. This arrest of these buds is due GOEBEL says "to all the available food material being devoted to the devel-

opment of one terminal bud." Many tuber- and bulb-forming plants do not normally produce seeds, but, as in *Lilium candidum*, *Lachenalia luteola*, etc., if the bulbs be cut away and prevented from forming, seeds will be produced. "In the normal condition," GOEBEL says, "seed formation is hindered because the plastic material which might be used for seeds streams into the bulb." The inference is that this material, prevented from going to the bulbs, will flow to the seeds. This conception plays a fundamental part in GOEBEL'S explanation of how the removal of one part may start the development of another. SACHS (**10**) gives a similar explanation for the development of the cotyledonary buds in Phaseolus, stating that it is due to increase of food resulting from the removal of the main axis. To demonstrate this it must be shown that these lateral buds, though in intimate contact with large cotyledons, remain undeveloped because of lack of food, and further that when the terminal bud is removed they will not develop if the possibility of an increased amount of food is precluded.

FIG. 4

The attempt is made in the following experiments to determine this experimentally. The plants used, unless otherwise stated, were seedlings, the epicotyl varying from 1 to 9^{cm} long, with the first pair of leaves not yet fully opened, and the cotyledons still full of food (*fig. 4*).

If an increase of nutritive material in the axial primordia, due to the removal of the parts using this while the source of supply is still active, is the cause of development of these structures, then while removing the central shoot, if we at the same time remove the source of the food, there should be no development of the primordia.

Experiment 1.—Twelve plants were used, the epicotyl was cut

off about the middle and the cotyledons left intact. The axial buds developed in all (*fig. 3*). This was repeated later for different purposes on scores of plants, and the result was always the same, except that occasionally only one shoot would develop.

Experiment 2.—Twelve plants were used, the epicotyl was cut off, and one cotyledon was removed. In all cases shoots developed from both axils.

Experiment 3.—Seven plants were used, the epicotyl was cut off, and both cotyledons were cut off but left in their natural position so as not to introduce other factors, as light, air, or moisture from the soil. All developed shoots from both axils. This was repeated on many plants with similar results.

Experiment 4.—Three plants, almost mature, 40–50^{cm} high and with 6–8 internodes, were used. The cotyledons had been used up and had dropped away; the lower part of the stem had become quite hard, almost woody, and hollow. The plants were cut off a few centimeters above the ground, and five of the six primordia developed shoots.

Here as in the other three experiments it is clear that food derived from the cotyledons is no essential part of the stimulus which causes the young buds to develop. In the next three experiments the attempt was made to remove the terminal bud and at the same time diminish the nutritive supply of the other buds even to the point of starvation.

Experiment 5.—Five plants that had been grown with their roots in tap water were transferred to distilled water. All the cotyledons were cut off and also the epicotyl. All five of the plants slowly but completely regenerated. Here all supply of food from the substratum, from the cotyledons, and from the leaves is removed. In this experiment and others a large number of check plants showed that the cutting away of the cotyledons had no effect on the young buds if the primary axis was left intact.

Experiment 6.—Four seeds just germinating were taken, the radicle being 4^{cm} long, and the young stem with its leaves being still between the cotyledons. The cotyledons were forced open and both cut off, leaving only the small stem and root. The plants were then placed with the root in distilled water in the dark, and left for two

days. Both stem and root elongated rapidly, at the expense of the food in the young plant to start with, so that part, probably most, of the food was used up, and the rest was distributed throughout the now much larger plant. Then the stem was cut off as close as possible to the buds, and two of the plants were placed in the dark and two in the light. All four regenerated slowly.

Experiment 7.—Twelve young plants were used as follows: *a*, two were cut off between the point of attachment of the cotyledons and the buds in their axils, so as to cut away the cotyledons and hypocotyl, leaving epicotyl with apex intact and primordia at the base, and set in a moist chamber; *b*, from ten plants the cotyledons were removed and the stem cut off close above the primordia, then the hypocotyl was cut off immediately below the primordia, leaving a small piece of the stem (average 8^{mm} in length) with the primordia attached; five of these were set in darkness and five in light in a moist chamber, with the base of each piece resting on wet filter paper. In *a* there was no development of the buds; in *b*, of the buds in the dark those on four plants grew to be 1–2^{cm} long and then died, presumably from starvation; while those in the light developed slowly at first and faster as they formed chlorophyll, finally forming shoots.

These experiments show conclusively that when the young shoot is removed the removal also of the food supply does not hinder the development of the buds at the base, and that it occurs when there is not only no increase in the food, but when the primordia and the surrounding parts are in a condition of starvation. Plants were grown in the most favorable conditions of rich soil, inorganic nutrient solutions, light, and moisture, so that vegetative growth was luxuriant, but only the removal of the apex had any influence in inciting the buds below to growth. The constant factor in every case is the removal of the apex, and neither an increase in food dependent on the removal of the growing shoot, nor any decrease that might occur in the nutritive relations constitute an essential part of the stimulus.

In Bryophyllum GOEBEL (**3**, p. 420) says that the vegetative points serve as "centers of attraction for the constructive materials." Those on the shoot, according to him, because of their more direct connections with the conducting system act as stronger attractive centers than those on the leaves. Of the buds on the shoot the

terminal one exerts a stronger attraction than the lateral ones. If we cut off the terminal bud the lateral ones develop, and GOEBEL (**3**, p. 418) has shown that if we cut off all the shoot buds those on the leaves develop. His explanation is that the stronger points of attraction being no longer active, the "building material" is drawn toward the points of "weaker attraction," *i. e.*, the growing points of the leaves.

Such a conception is scarcely in harmony with the principles that control the movement of materials in plants. Any substance in solution in the plant necessarily follows the general laws of solutions, and will diffuse toward any point only when there is less of it there than at the place from which it moves. A movement of constructive materials from the leaves or cotyledons only occurs when there is more of it in solution there than elsewhere, and if the flow is directed toward the terminal bud it is only because that is the point of least concentration. If at any time there were less in the lateral buds than in the terminal one, it would diffuse into the former. The fact of any food material moving toward the terminal part and past the lateral buds is positive evidence that the terminal bud contains less of this in solution (and it is only in solution that it would be available) than the others. There is no evidence for assuming that the food is "attracted" into certain buds in sufficient quantity to start growth and not into others; for until growth starts all will contain an equal amount, and afterwards, if food continues to move toward certain buds and not toward others it can only be because growth (or some other cause) keeps the amount in the former constantly less than in the latter.

Thus there is no ground for the supposition that the buds that develop in regeneration are any better supplied with food after they start to sprout than before, and that when they do not develop, as GOEBEL'S hypothesis implies, it is because of lack of food. Frequently they are stored with food and will begin to develop so soon as isolated from the parent plant. Starvation, as we know, will not cause growth to cease until it is far more severe than it is ever likely to be on any well-nourished plant; and, as MORGAN (**7**, p. 27) has pointed out, animals regenerate even while starving to death.

Experiment 8.—From young shoots of Salix and Lycium the ter-

minal bud and all the leaves were removed, and the shoots, separated from the parent plants, were placed in the dark. The axillary buds started to develop and continued until all the nourishment in the shoots were exhausted.

Roots of Taraxacum were cut into several pieces, and on each piece new shoots arose. We cannot say that each piece was either better or more poorly nourished than before. A Marchantia thallus grown in very weak light and plainly in a semi-starved condition regenerated when cut across transversely.

It seems clear from the experimental evidence that, at least in the plants mentioned, there need be no increase in nutritive conditions to occasion regeneration, and we must look elsewhere for the stimulus.

DISTURBANCE IN WATER CONTENT.

When the leaves or other transpiring surfaces of a plant are removed, there is often opportunity for a better supply of water in the remaining parts. The profound influence of water as a factor in growth is too well known to need emphasis. If the leaves of many trees or shrubs be removed early enough, the axillary buds will develop shoots instead of remaining dormant until the next year. DeCANDOLLE (**1**) attributed this to the "sap" being no longer drawn away from the buds by the leaves. WIESNER (**15**) thinks that the young buds are hindered from developing because the water is withdrawn from them by the more actively transpiring leaves. In isolated pieces of Salix stems VÖCHTING (**13**) believes that water is the factor that determines the appearance of roots, and KLEBS (**5**, p. 104) had experimentally proved this to be true in at least some species. WAKKER (**14**) obtained buds on leaves of Bryophyllum by submerging them, and thinks it due to disturbances in the water current. GOEBEL (**3**, p. 393) obtained buds on leaves of *Aneimia rotundifolia* by either submerging them or placing them in very moist air; also on uninjured leaves of *Cardamine pratensis* by keeping the plants in moist air. KLEBS attributes this to the checking of transpiration and consequent abundance of water in the leaf. By surrounding a portion of an uninjured stem of Salix by a glass cylinder filled with water, KLEBS (**5**, p. 104) found that roots develop upon that part. Other plants, especially those whose natural habitat is in wet places,

possess this ability to produce roots along portions of the stem that are kept wet. Sometimes, as in *Veronica anagallis*, the root primordia are already laid down, but in most plants these are not present. KLEBS believes that in these cases the development of the roots is due to the increased absorption of water, and holds this factor to be of great importance in other regeneration phenomena. The following experiments will throw light upon this.

The question was attacked by two opposite methods: the effect was determined of (1) growing plants without injury or any removal under such conditions as would increase the water in the plant to the highest amount possible; (2) of supplying the other conditions for regeneration and at the same time decreasing the water content of the plant to the least possible amount compatible with actual existence.

Experiment 9.—Six plants in pots were placed under bell jars, whose inner surfaces were lined with wet filter paper. The plants grew rapidly, but no regeneration occurred.

Experiment 10.—The same experiment was repeated with five other plants, with the same result. While this usual method of forming a moist chamber gives an atmosphere that minimizes transpiration, it does not altogether inhibit it. This objection was overcome in two ways (experiments 11 and 17).

Experiment 11.—Two plants were set in a large bell jar, and this was set over a vessel of water so that the bottom of the bell jar was just below the surface of the water. This water was heated to and kept at a constant temperature of 33°. The air of the greenhouse averaged 18–20°, while that in the bell jar was 24°, a good growing temperature for this plant. The air surrounding the plant was thus saturated from a water surface whose temperature was 7° higher than the temperature of the plant, which entirely inhibits any evaporation from the latter. The plants grew rapidly, but the primordia at the base showed no signs of developing. Here the plant is undoubtedly saturated with water.

Experiment 12.—Attempts were made to prevent transpiration by covering the leaves with vaseline, cocoa butter, or soft wax, but in no case was the development of the buds produced.

Experiment 13.—In the moist chambers described the whole plant was in the moist air. Two plants were next arranged, each in a glass

cylinder, so that the lower part of the stem of each plant passed through a rubber stopper inserted in the bottom of the cylinder. Thus the stem and foliage were in the cylinder, the cotyledons were below in the air, and the roots hung down into a nutrient salt solution. The cylinder was lined with wet filter paper and covered at the top. The plants grew rapidly, but the basal primordia did not develop.

Experiment 14.—Three other plants were arranged in the same way, except that the cylinders were filled with water instead of moist air. The stems grew rapidly for a few days, then the growth gradually decreased, and finally ceased entirely. No development of the basal buds occurred until growth ceased, when they developed. This was repeated by inverting three plants with their foliage in a large aquarium, with the same result. When the shoots were killed by the prolonged submergence, however, the basal buds developed shoots.

Experiment 15.—All the foliage was cut from five plants, thus removing the transpiration surface, and no development of basal buds followed. All the leaves and also the cotyledons were removed from three other plants, with the same result.

Experiment 16.—Five large plants, with six to eight internodes and a large display of foliage were selected. The vegetative tip was removed from each and also the buds in the axils of all the leaves. In four of them the basal buds developed. Here the disturbance in the water content could only have been trifling, for careful determination before and after cutting off the tips showed no appreciable diminution in the amount of transpiration.

Experiment 17.—A second moist chamber that kept the plant saturated was formed in the following way: a glass tube was drawn to a fine end with a capillary opening and through this a fine jet of water was forced, which struck a small piece of ground glass held obliquely to it and was scattered into a fine mist. In this mist five plants were set, the fine spray continuously settling on every part of the plant, not only the upper but also the under surface, for it circulated freely on the slight air currents always present. Here the plants were covered constantly with a thin layer of water, which completely checked transpiration, but the excellent aeration allowed a healthy growth. All the plants grew rapidly, but there was no development of the buds at the base.

The experiments thus far show that the maximum amount of water in the plant will not induce the basal primordia to develop. The opposite method was then tried, *i. e.*, supplying the other possible factors in regeneration and at the same time withdrawing water from the plant.

Experiment 18.—Eight plants were used, the soil allowed to dry until the leaves wilted, and the stems then cut off a few centimeters above the ground. No more water was added and the soil became quite dry. The remaining part of the stems wilted and the cotyledons began to shrivel. In spite of this the primordia developed on six of the plants; on the other two they started but soon withered completely. The six that developed grew very slowly and apparently suffered severely from lack of water. Three of them were then watered, and at once recovered turgidity and grew normally. On the other three the young shoots reached an average length of 3^{cm} and then died. Here the buds started to develop with a turgidity much below the normal, and continued slowly against a decreasing turgescence until the death point was reached. This part of the experiment was repeated on several occasions and always with the same result. Check plants under the same conditions, but with the stems not removed, showed no development of the buds. The following more accurate method was then used.

Experiment 19.—To the weak nutrient salt solution in which the plants were frequently grown was added KNO_3 to make up solutions of the following gram-equivalent proportions: $\frac{2}{3}$, $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{4}$, $\frac{1}{5}$, $\frac{1}{6}$, $\frac{1}{7}$, $\frac{1}{8}$, $\frac{1}{9}$, $\frac{1}{10}$. To each of these were transferred plants whose roots had been grown in the usual nutrient salt solution. In four days the roots in the $\frac{2}{3}$, $\frac{1}{2}$, and $\frac{1}{3}$ gram-equivalent solutions were entirely plasmolyzed and killed, and the stems and leaves wilted. In the $\frac{1}{4}$ solution the roots were partially plasmolyzed, though not killed, and the leaves were just flaccid. In the $\frac{1}{5}$ solution the roots seemed quite healthy, and also the shoots, but growth was very slow. In all the other solutions the plants were vigorous and grew rapidly. Another set of these solutions was made up, and in each were placed plants with vigorous roots that had been developed in nutrient salt solutions, and each stem was cut off. As before, those in the $\frac{2}{3}$, $\frac{1}{2}$ and $\frac{1}{3}$ solutions were killed by plasmolysis; in the $\frac{1}{4}$ solution the

roots at the end of the week were killed, but the stem was still alive, though wilted, and the buds were developing; the cotyledons were much shrunken from loss of water which had been drawn out by the strong solution. The plants lived for several weeks, the young shoots slowly developing, and then died as the solution became stronger through evaporation. In the $\frac{1}{5}$ solution the development of the buds was slow but normal. In all the others it was quite active.

Experiment 20.—The nutrient salt solution mentioned was made up in concentrations which approximate the following: $\frac{25}{100}$, $\frac{30}{100}$, $\frac{35}{100}$, $\frac{37}{100}$, $\frac{40}{100}$, $\frac{45}{100}$, $\frac{48}{100}$, $\frac{50}{100}$, $\frac{55}{100}$, $\frac{60}{100}$ gram-equivalent solution. These concentrations are only approximate, for probably ionization is not complete in any, certainly not in the stronger solutions, and also sufficient KOH was added to make them neutral. As it is only the relative strengths of solution that are required, it was not necessary to determine the actual osmotic pressure. One plant was used for each solution, and as before the roots were in the solution and the cotyledons and the rest of the plant in the air. Each plant was cut off a few centimeters above the cotyledons. In the three strongest solutions the plants were entirely killed, wilting very rapidly. In the $\frac{43}{100}$ gram-equivalent solution, after two weeks the roots were still alive but very much twisted and contorted, and so far as could be seen no growth had occurred in them. The development of the buds was slow but complete. In the next solution below this the growth of the buds was considerably faster, and in all the others it was normal. Similar plants with their shoots and leaves intact were put in the $\frac{45}{100}$ and $\frac{48}{100}$ solutions and in two days the leaves had wilted down.

Experiment 21.—The last experiment was repeated by cutting off not only the stem but also the cotyledons. The result was the same, new shoots slowly forming while the water was being withdrawn.

The result of these experiments shows (1) that the buds in the axils of the cotyledons will not develop under the influence of maximum turgidity of the cells so long as the upper part is still functioning, and (2) that if the stem be cut off the buds will develop against a partial plasmolysis of the plant. With the shoot intact no change in the water content will start the buds into activity, so that the stimulus to their development does not need to include any change in the water content of the cells. The factor that is constant in all the cases is the removal of the growing points above.

If WIESNER's conception that checking transpiration and consequent accumulation of water is the cause of the development of buds when the leaves are removed is correct, we should expect to find the same development if we occasion an equal accumulation of water without the removal of the leaves. But this does not happen. Experiments were conducted on young shoots of Salix, Populus, Cornus, Ulmus, Solidago, Silphium, and other plants. These need not be described individually, it being sufficient to say that when grown in the moist chambers described above, including the one in which the chamber was filled perpetually with fine spray, the buds showed no tendency to develop. In some cases cuttings were used, and in others plants with roots in soil or in nutrient water cultures. No loss of water was possible anywhere, and every part of the plant capable of taking in water was doing so. On the other hand, as will be discussed later, the buds promptly develop even when very plainly suffering from lack of water, if the tip of the shoot be removed.

Roots of Taraxacum with all buds removed were left in rather damp air, but yet allowing a slow evaporation of water from the surface. While thus slowly drying they all regenerated new buds and shoots. WAKKER obtained buds on leaves of Bryophyllum when submerged, but, as GOEBEL has mentioned, we do not know what other factors come into operation in the course of such drastic treatment. One of the most striking cases of the direct influence of water in inciting regeneration is that of *Cardamine pratense*, which, as GOEBEL (**3**, p. 425) showed, produces shoots on the leaves while still intact, when the plant is placed in a moist chamber. Bud primordia in this case are already formed on the leaves, and in their moist shady habitat in nature, where vegetative growth is luxuriant, develop abundantly, so that here we are probably only dealing with the usual precocious vegetative growth of this plant.

It is in the case of root production that we get what at first seems to be the most striking examples of the direct influence of water on the origin of new parts. It is well known that many stems form roots when cut off and placed in water, and KLEBS, as mentioned above, has shown that in Salix the application of water to local areas of the stem, without any wounding, is followed by a copious appearance of roots. But even here a closer analysis of the condition may

reveal other factors than those which have been considered the most important. Experiments on this connection are to be mentioned. KLEBS (**5,** p. 109) says that when through wounding or separation roots or shoots either infold themselves or are produced entirely anew, it is because through this separation just those conditions are brought about in the cells which under all other circumstances would start into operation the same "building processes;" and this condition in Salix he asserts lies in the necessary accumulation of water in the parts concerned. Experiments in this connection are to be mentioned. VÖCHTING (**13**), in his classic experiments on Salix, obtained roots on pieces of stem when placed in moist air, and concluded that the cause of root development was the increased moisture. This assumes that the stems absorbed moisture from the atmosphere, and also that if they had not absorbed this the roots would not have developed.

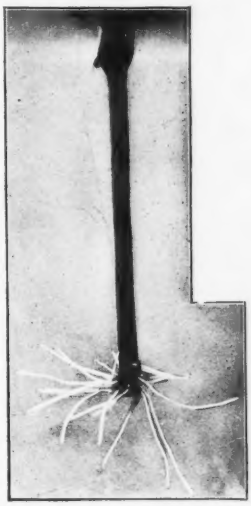

FIG. 5

Before we can attribute a result to any factor it is necessary to show (1) that that factor is always present when the result occurs, and (2) that when it is absent the result will not occur. Will roots of Salix, for example, develop on the stem only after "the necessary accumulation of water;" or can we produce them without this, or with even a decrease of water in the parts producing them? The following experiments were to determine this. For convenience, those on Phaseolus will be described first. If the stem of this plant be cut off anywhere and placed in water, roots come out abundantly at the lower end (*fig. 5*). These arise from the pericycle entirely anew, no root primordia existing anywhere on the stem.

Experiment 22.—Entire plants, with roots in soil or water cultures, were placed in the three moist chambers described above. They grew rapidly and no roots formed on the stems. There can be no doubt that the stems were entirely saturated with water.

Experiment 23.—Plants with roots grown in water culture were placed in water so that the stems were submerged. From several the cuticle and outer part of the cortex was peeled off, so that water would enter freely, and in some the foliage was enclosed in a moist chamber. No roots developed.

Experiment 24.—Stems with roots intact were submerged as in the last experiment, but some of the stems were slit through the middle longitudinally, while from others a slice out of the side the length of the internode and one-third the diameter was taken. Thus free

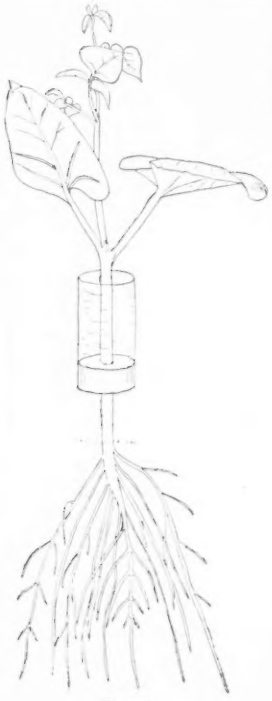

FIG. 6

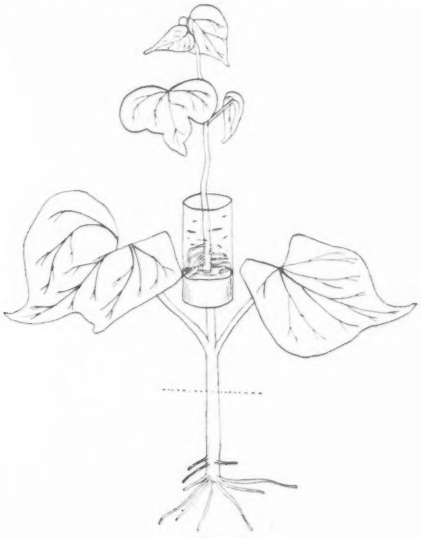

FIG. 7

absorption of water was possible all along the stem, but no roots developed. If, as will be discussed again, the cut be a transverse one, severing some of the bundles, roots promptly develop just above this; or if the roots be removed new roots form along the stem.

Experiment 25.—Stems with roots intact below were surrounded at local regions by water in glass cylinders (*fig. 6*), and the cortex was removed so as to allow free absorption. No roots developed. If, however, as in *fig. 7*, stems were used from which the root

system had been removed, roots promptly came at these watered areas.

These experiments show that contact with or the free absorption of water by the stem, or the complete saturation of the stem and whole plant, will not induce root development on the stems when the roots below are intact.

FIG. 8

On the other hand, the roots may develop when the parts from which they arise not only do not absorb any water, but are actually wilting. From a considerable number only four experiments will be mentioned.

Experiment 26.—Plants were cut off near the base and the whole plants placed in damp air, the lower free end of the stem being suspended in the air and not in contact with water. A slow transpiration necessarily occurred and the plants gradually wilted. The lower end of the stem became quite dry, yet from it roots arose.

Experiment 27.—Three pieces of internodes were placed in damp air. They wilted until there was a conspicuous shrinkage, and yet at the basal end of each roots developed. The weight of the pieces at the beginning of the experiment was 8.7^{gr}, and eight days later, when roots had just appeared, 7.9^{gr}.

FIG. 9

Experiment 28.—Stems were cut off near the base, and a portion of the upper part of the internode was surrounded by water, as shown in *fig. 8*. The lower part projected downward through a hole in a glass plate and was in the rather dry air of the laboratory; while over all the rest of the plant was placed a bell jar to keep the air moist so that the plant would not wilt. Roots soon came out from the part of the stem surrounded by water. The basal end projecting down into the air became somewhat wilted, especially toward the base where the end for about 1^{cm} was completely dried up. In spite of this, roots formed just above this dried portion, and broke through the epidermis but could not continue in the dry air.

Experiment 29.—Stems were cut off and the lower part placed in water. Above the water a deep notch was cut in each stem. Roots developed abundantly at the lower end in the water, and also just above the notch. The vessels being cut off from the water supply, the tissues were quite wilted and shrunken. In the dry air the roots did not elongate more than 1–2^{mm}, but if the air is moist they grow vigorously (*fig. 9*). If, as stated, a portion of the stem is surrounded by water, no roots appear on this part so long as the roots are intact below (*fig. 6*); but if the latter are cut off, roots appear at the former place as well as at the place below from which they were removed. But the cutting off of the root system in this case cannot influence the amount of water in the stem, unless it be to diminish it.

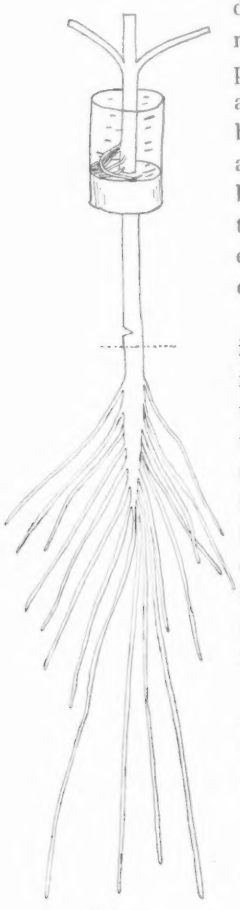

FIG. 10

Experiment 30.—The plant was fixed as shown in *fig. 10.* Below the cylinder of water surrounding the stem a notch was cut about one-third the way through the stem. This severed the connection with the root, along these bundles, from this point upwards. Roots appeared in the water above, on the side directly above the notch, and from the bundles severed by it. Here again, if the cutting of the notch had any effect on the amount of water in the part of the stem directly above it, it would only be to diminish it, yet its effect was to produce roots there.

Experiment 31 (*fig. 11*).—Both portions *a* and *b* are in the water, and if there is any difference *a* has the better chance of becoming saturated; yet *b* alone produces roots.

Experiment 32 (*fig. 12*).—The apical end is inverted in water and the basal in somewhat damp air, but allowing considerable evaporation. No roots at all come on the former, but on the latter many primordia are formed and break through the cortex, and a few grow out into the air. If the air be kept saturated, or the end surrounded by water, many roots grow out vigorously.

These experiments all show, at least in the bean, that an increase of water at any point along the stem will not in itself incite the formation of roots, and that root primordia will be organized when the cells there contain much less water than when growing normally. Their subsequent development depends on sufficient external moisture to prevent them from wilting.

In Salix, root primordia are laid down early along the stem in the vicinity of the buds. In some species, at least, contact with water, as KLEBS has shown, will incite these to active growth. It does not necessarily follow, however, that this is due to the increased absorption of

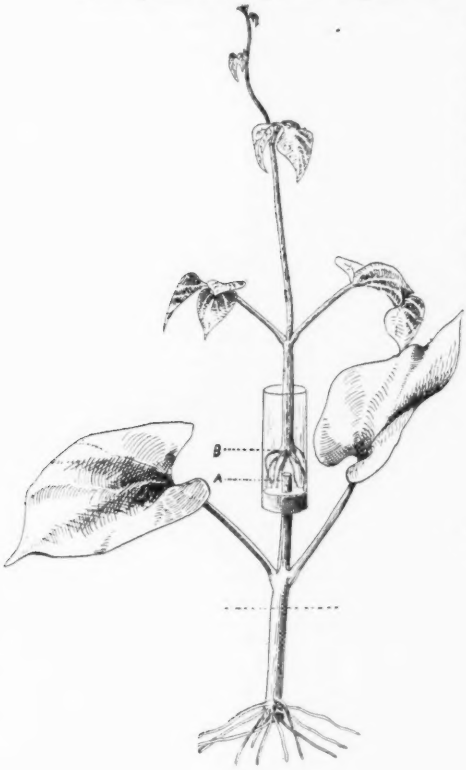

FIG. 11

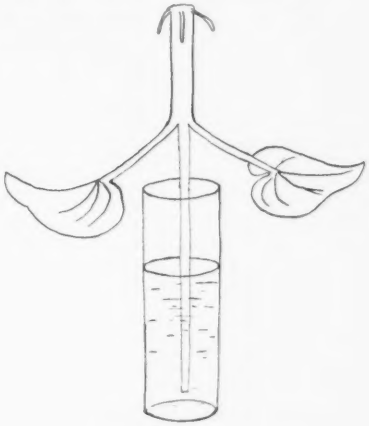

FIG. 12

water by these cells, as KLEBS maintains. The following experiments throw some light on this.

Experiment 33.—Three pieces of *Salix glaucophylla* stem two years old were cut off from plants which were growing in pots in the greenhouse and weighed. The aggregate weight was 27.5^{gr}. These were placed horizontally in a chamber where the air was just moist.

In less than a week roots had appeared on all three near the base. The weight now was found to be 26^{gr}, a loss of 1.5^{gr}, most of which certainly was water. Another piece at first weighed 10.5^{gr}; and when the roots coming out on it were 1^{cm} long the weight was 10.1^{gr}. Thus, with the piece as a whole losing water, but with a moist atmosphere outside, roots develop.

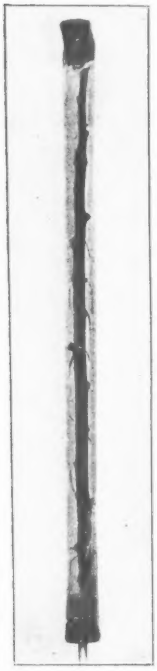

FIG. 13

Experiment 34.—Two pieces of stem 30^{cm} long were fastened with their basal ends connected with the water faucet, so that the water was forced into them under high pressure. This pressure was sufficient to cause water to ooze slowly from the opposite end of the stem. The air surrounding the stem was that of the laboratory. No roots anywhere enlarged enough to break through the cortex to the surface. Here a greatly increased amount of water in the stem does not start the roots.

Experiment 35.—Stems growing with vigorous roots in water cultures were selected. A few inches above the water a ring of bark 5^{mm} wide, cutting into the wood, was removed. Just above this for about 1^{cm} the bark (*i. e.*, all outside of the wood) was plainly drier than at any other point, yet the root primordia in this part in a few days enlarged and broke through the cortex and epidermis, but went no farther; but when one of the pieces was put in moist air they grew out rapidly.

Experiment 36.—Five pieces of stem of *Salix fragilis*, each 30^{cm} long and one and two years old, were cut off from larger branches growing with roots in water. Two of the pieces bore young leaves, while on the other three the buds had not yet opened. Each piece, carefully freed from any water on the surface, was placed inside a dry glass tube as small in diameter as would admit the piece (*fig. 13*). Each tube was sealed air tight at each end by a rubber stopper and wax. Here there was no possibility of any absorption of moisture; on the other hand evaporation was constantly going on from the surface of the stems, and the moisture condensed in little droplets on

the inside of the tube. The air in the tube under these circumstances must have been saturated with moisture, all of course at the expense of the water in the stem. Within a week three of the stems and a few days later a fourth showed vigorous roots coming out, which grew rapidly and soon were several centimeters long. Here again the stimulus certainly was not any increased water in the stems. It seems as though a moist atmosphere outside of the stem can act as a stimulus without any increase, in fact even a decrease, inside. How this could act through the epidermal and outer corky layers is not clear. At first it seemed that the real cause lay in the removal of the piece from all influence of the roots below, but glass tubes similarly placed around portions of longer pieces whose roots were intact and active below resulted in the production of roots just the same. Similar pieces covered with a thin coat of wax to prevent any evaporation showed no signs of root development.

FIG. 14

In submerged aquatics, where there is no current of water through the plant, but where the absence of a cutinized epidermis allows free diffusion in and out at every point, and where all the cells are constantly saturated, the removal of a part of the stem does not cause any change in the amount of water present. If such plants regenerate it is not due to disturbances in the water content.

Experiment 37.—Portions of the stems of the extreme aquatic forms of *Proserpinaca palustris* and *Ranunculus multifidus* were severed from the parent plants and left submerged. In all cases new roots were at once organized and grew rapidly at or near the basal end, and at the other end shoots started from the latent buds in the leaf axils (*fig. 14*). Isolated pieces of roots of Taraxacum, *Rumex crispus*, and stems of Zamia all organized new shoots while they were still losing moisture. In cases like Salix there is no doubt that contact with water will start the development of roots along the stem, yet these can also be started by other causes while the cells are losing

water. In the great majority of instances where regeneration occurs, however, it cannot be due to any disturbance in the amount of water present in the parts concerned. Several leaves of Begonia and Bryophyllum were kept in the air of the room, but with their petioles in water. The blades quite plainly were not more turgid than those left on the plants, and not so much so as those on plants grown in moist air; yet they produced buds while the latter did not.

The results of experiments with the other factors mentioned will be presented in the second paper of this series.

THE UNIVERSITY OF CHICAGO.

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ON PROTEOLYTIC ENZYMES. II.

ARTHUR L. DEAN.

SINCE the writing of the previous paper[1] on the vegetable proteolytic enzymes, an article by VERNON[2] has appeared in which he gives an account of his investigation of the distribution of erepsin in the animal body. Comparative tests were made of glycerin extracts from thirteen different tissues of the cat, eight of the rabbit, eight of the guinea pig, seven of the pigeon, eight of the frog, seven of the eel, six of the lobster, and three of the fresh water mussel. In every case an erepsin was found to be present. The comparison of the activity of the various extracts was effected by means of colorimetric estimations of the intensity of the biuret reaction after certain periods of digestion. The tissue extracts from the warm blooded animals were more active than those from the cold blooded animals; the extracts from the invertebrate tissues had a relatively slight action. In the warm blooded animals it is not the intestinal mucous membrane which is richest in erepsin, but the kidney in the cat, rabbit, and pigeon, and the pancreas in the case of the guinea pig.

It might be noted that VERNON gives no record of experiments made with the various extracts to determine whether or not they are incapable of digesting the proteids of the tissues in which the enzymes occur. His experiments, being all conducted with Witte peptone solutions, do not conclusively show that the enzymes whose activity was observed are incapable of acting on any proteids except albumoses and peptones.

In a former communication evidence was given to show that *Phaseolus vulgaris* contains in its seeds a fairly active proteolytic enzyme. No action of this protease on the proteids of the seed could be demonstrated. Its power to act on Witte peptone as a whole, and on the albumose fractions separated from it, could be readily shown. Moreover, as germination progressed the cotyledons at all stages contained this ereptase and at no period of germination could

[1] DEAN: BOT. GAZETTE **39**:321. 1904.

[2] VERNON: Jour. Physiol. **32**:33. 1904.

any evidence of a tryptic enzyme be obtained. Further studies have been made of this seed enzyme in the hope of throwing light on the processes occurring during germination.

FURTHER EXPERIMENTS WITH THE GERMINATING SEEDS OF *PHASEOLUS VULGARIS*

The following experiment was carried out to demonstrate the action of the seed ereptase of the bean on the proteoses to be obtained from phaseolin, the principal globulin of the seed. Phaseolin was prepared according to OSBORNE'S[3] method from white medium field beans, the yield from two kilograms of beans amounting to something over 75^{gm}. A small amount of phaselin was also obtained by the alcoholic precipitation of a part of the solution from which the phaseolin had separated on dialysis. This albumin gives a marked Adamkiewicz reaction.

By mixing a known weight of phaseolin with fifty times its weight of 2 per cent. sulphuric acid and heating for three and a half to four hours in a steam sterilizer, a slightly brown gelatinous fluid was obtained having the odor characteristic of acid proteid hydrolysis. This fluid was cooled, diluted with water, and the gelatinous antialbumid removed by filtration. The filtrate from the antialbumid was neutralized with barium hydroxide and the filtrate from the barium sulphate concentrated and treated with several volumes of alcohol. The mixture of proteoses obtained in this way was collected on a filter, washed with absolute alcohol and ether, and dried *in vacuo* over sulphuric acid.

To obtain an ereptase solution finely ground beans were extracted with water and the extract filtered through pulp filters until nearly clear. Ten cubic centimeters of this solution were placed in each of four dry test tubes and two of the portions boiled. Solutions of Witte peptone and phaseolin proteoses were prepared by dissolving 0.2^{gm} of each preparation in 25^{cc} of water. The test solutions were made up as follows:

1. 10^{cc} enzyme solution + 10^{cc} phaseolin proteose solution + 9 drops of toluol.
2. Same as no. 1, using boiled enzyme solution.
3. 10^{cc} enzyme solution + 10^{cc} Witte peptone solution + 9 drops of toluol.
4. Same as no. 3, using boiled enzyme solution.

[3] OSBORNE: 17th Annual Report of the Conn. Agric. Experiment Sta., Part IV. 186. 1893.

The corked tubes were kept in the incubator for six days. At the expiration of that time each digestion was boiled, acidified with a couple of drops of acetic acid, and filtered. Ten cubic centimeters of each filtrate were removed to a clean tube, and, after the addition of 5^{cc} of 10 per cent. sodium hydroxide solution, dilute copper sulphate solution was added to the maximum biuret reaction. After standing for several minutes the intensity of the biruet reactions was compared. The comparison showed that a marked digestion of both proteose mixtures had taken place, the one from phaseolin being rather more vigorously attacked. The unboiled digestion with Witte peptone gave a tryptophan reaction with bromine water, the other unboiled digestion did not.

Several trials were made of the digestibility of the acid phaseolin prepared by heating phaseolin for a few minutes with dilute sulphuric acid. The results obtained indicated that the enzyme of the seed had, at the most, but a feeble action on this body.

Various observers have shown that the antiseptics used in enzyme experiments may exert an inhibiting effect on the action of the ferment. VINES demonstrated that in papain digestions where sodium fluoride is used the action goes but little beyond the stage where albumoses and peptones are formed; whereas with other antiseptics, hydrocyanic acid for example, a marked formation of amido-acids takes place as shown by the production of tryptophan. It might be argued that the toluol used throughout the experiments with Phaseolus had an inhibiting action on the enzyme, so that it was unable to attack the proteids of the seed and could only act on albumoses and peptones. The two following experiments were carried out to settle that point:

Experiment I.—Thirty-five grams of finely comminuted cotyledons from six-day old bean seedlings were extracted for one and a half hours with 175^{cc} of water. The extract was filtered nearly clear through pulp filters and then forced through a Pasteur-Chamberland filter into a sterile flask. Three portions of 25^{cc} each were measured with a sterile pipette into three small sterile flasks. To no. 1 nothing further was added; to no. 2 was added 0.07^{gm} of sterile citric acid, making the solution $\frac{N}{25}$ citric acid; the contents

of no. 3 were boiled. The cotton plugs in the flasks were replaced corks. After keeping the three flasks in the incubator for forty-one hours, 20^{cc} of tannic acid reagent (7 per cent. tannic acid in 2 per cent. acetic acid) were run into each. After standing a few moments the contents were filtered through dry filters and duplicates of 20^{cc} from each filtrate analyzed for nitrogen by the Kjeldahl method.

Analyses of filtrates from no. 1 gave 0.0036^{gm} N
Analyses of filtrates from no. 2 gave 0.0036^{gm} N
Analyses of filtrates from no. 3 gave 0.0033^{gm} N

Experiment II.—Another extract from cotyledons of six-day old seedlings was prepared by treating 28^{gm} of finely ground tissue with 140^{cc} of cold water for three hours. The extract was filtered as in the previous experiment and 25^{cc} of the filtrate removed into two sterile flasks. The contents of one flask were boiled and both were corked with sterile corks and kept in the incubator for three days. To test the ereptic activity of the bacteria-free filtrate 15^{cc} were placed in a test tube with 0.5^{gm} of Witte peptone and a little toluol. At the end of the digestion period the fluid in this tube was found to give a strong tryptophan reaction with bromine water, showing that the enzyme had not been held back by filtration through porcelain. The contents of the two flasks were treated as in the previous experiment:

Analyses of filtrate from unboiled digestion gave 0.0036^{gm} N.
Analyses of filtrate from boiled digestion gave 0.0030 ^{gm} N.

The differences between the boiled and unboiled digestions in these two experiments are so slight as to be within the limits of error of the method used and cannot be taken to show any hydrolysis of the proteid in the cotyledon extract.

PROTEOLYSIS DURING THE GERMINATION OF *PHASEOLUS VULGARIS*

We have every reason for believing that the germination of the bean is accompanied by a hydrolysis of the proteids therein contained, a hydrolysis which our experimental evidence leads us to conclude must be started, at least, by some other agency than an enzyme. That proteolysis does accompany germination of this seed is shown by the following experiments, where the amounts of

coaguable and non-coaguable nitrogen were determined in the seeds and in the cotyledons of young seedlings.

About 75^{gm} of beans were soaked in water and the cotyledons separated from the skins and embryonic plants. The cotyledons after being washed were dried at 60° C. and ground in a hand mill, yielding the preparation *A*. Another part of the same lot of beans was planted in the greenhouse and allowed to germinate for seven days. At the end of this time the somewhat shrunken cotyledons had been pushed above ground, had begun to turn green, and were separating to allow the plumules to push out. These cotyledons were removed from the plants, washed, and dried at 80° C. When dry they were ground in the mill, giving preparation *B*. Portions of both *A* and *B* were rubbed to the finest powder possible in the mortar and dried at 100° C. to constant weight. Duplicate portions of 1^{gm} each of *A* and *B* were then weighed into small beakers, 15^{cc} of water added, and, after bringing to a boil, 2 drops of 10 per cent. acetic acid were stirred in. After allowing the coagulated proteids to settle for a moment or two the contents of each beaker were transferred to a dry washed filter paper in a funnel held in a Kjeldahl digestion flask. The washing of the precipitates and their quantitative transfer to the filters was effected by the use of six portions of 5^{cc} each of boiling distilled water. After thoroughly draining, the filters with the contained precipitates were transferred to Kjeldahl flasks; the precipitates and filtrates were then analyzed for nitrogen with the following average results:

Total nitrogen in $A = 0.0407^{gm}$	= 4.17 per cent.
Total nitrogen in $B = 0.0396^{gm}$	= 3.96 per cent.
Percentage of nitrogen in *A* as coaguable proteids =	90.7 per cent.
Percentage of nitrogen in *B* as coaguable proteids =	61.4 per cent.
Percentage of nitrogen in *A* as soluble compounds =	9.3 per cent.
Percentage of nitrogen in *B* as soluble compounds =	38.6 per cent.

It is worth noting that in the germination of the bean the consumption of nitrogenous foods proceeds at practically the same rate as that of the non-nitrogenous materials stored there; as a consequence the percentages of nitrogen in the cotyledons in the various stages of germination are nearly constant. After the cotyledons have become emptied the percentage of nitrogen changes, since the cell walls of

the shriveled cotyledons make up so large a percentage of the dry weight.

The experiment described above shows that proteolysis actually does occur as a step in the process of the utilization of the stored proteid. It is of interest in this connection to see if this process occurs when the cotyledons are removed from the young seedlings and kept under sterile conditions. A series of preliminary tests showed that if the cotyledons of germinating beans are treated for twenty minutes with a 0.5 per cent. solution of mercuric chloride and then repeatedly washed with sterile water and kept in sterile tubes they will remain free from bacterial or fungal infection. The procedure kills the peripheral cells of the cotyledons, as shown by the fact that they do not turn green if kept in the light; whereas unsterilized cotyledons show a gradual development of chlorophyll under the same conditions of light and moisture. Comparative tests of the sterilized and unsterilized cotyledons showed that both contained ereptase, but that there was a noticeably smaller amount in the ones treated with mercuric chloride. It is probable that the poison not only killed the outer layer of cells, but also destroyed the enzymes contained in them.

Three-day old seedlings of Phaseolus were removed from the soil and the cotyledons separated. A quantity of these were washed, dried between 70° and 80° C., and ground in the mill, yielding preparation *C*. Ninety other perfectly sound cotyledons were selected, forty-five placed in each of two previously sterilized flasks, and kept covered with 0.5 per cent. mercuric chloride solution for twenty minutes. After pouring off the sublimate solution the cotyledons were washed five times with sterile water in quantities as great as that of the mercuric chloride solution used. In pouring off the last wash water sufficient was left in the flasks to keep the cotyledons moist. The flasks were closed with sterile corks and kept at room temperature—about 20° C. After three days one flask was opened and the contained cotyledons dried and ground, giving preparation *D*. The contents of the flask were judged to be sterile by the absence of any foreign growth and by the results of a transfer of a drop of the fluid from the bottom of the flask to a sterile agar tube. The second flask was kept unopened for five days longer and then the cotyledons in it were treated as the other portion, yielding preparation *E*.

Samples of preparations *C*, *D*, and *E* were finely ground, dried to constant weight and estimations of the coaguable and non-coaguable nitrogen were made in the same way as the similar determinations on *A* and *B*.

Total nitrogen in 1^{gm} of *C*	0.0426
Total nitrogen in 1^{gm} of *D*	0.0426
Total nitrogen in 1^{gm} of *E*	0.0432
Percentage of nitrogen in *C* as coaguable proteids	81.7
Percentage of nitrogen in *D* as coaguable proteids	88.0
Percentage of nitrogen in *E* as coaguable proteids	79.9
Percentage of non-proteid nitrogen in *C*	18.3
Percentage of non-proteid nitrogen in *D*	12.0
Percentage of non-proteid nitrogen in *E*	20.1

These results would indicate that under the conditions of the experiment there is no proteolysis occurring, although, as previously stated, the cotyledons still contain ereptase. A small increase in coaguable nitrogen was observed, followed by a rise to about the original amount. There seems to be no evident explanation for these small variations. Two facts may be noted: the oxygen supply to the living cells in the interior of the cotyledons was probably curtailed by the presence of the layers of dead cells on the surface; the correlation of the parts of the organism had been destroyed and the influence of that factor on the metabolic processes is not known. The results tend to emphasize the dependence of proteolysis in this seed upon the normal life of the organs.

DISTRIBUTION OF THE PROTEASES IN VARIOUS PARTS OF PLANTS OF *PHASEOLUS VULGARIS*

A large number of qualitative tests for proteolytic enzymes in various parts of the plants of Phaseolus were carried out, using the tryptophan reaction as an indication of proteolysis. It seems scarcely necessary to detail the methods and results in every instance. The experiments were conducted in a way analogus to those on the cotyledons in the various stages of germination, a full description of which was given in a former paper. In testing for a tryptic enzyme the tissues were sometimes allowed to autolyze without the addition of any further proteid, but in most cases edestin, phaseolin, or uncoagulated egg albumin was added. In searching for evidence of

tryptic action the filtrates obtained after coagulating the proteids of the digestion mixture were frequently tested with Millon's and the biruet reactions, as well as with the tryptophan reaction. The ereptic activity was tested with Witte peptone. The antiseptic used was toluol. Tests were made of the following tissues:

1. The embryonic plants in the seeds (exclusive of the cotyledons).
2. The hypocotyls and plumules from three-day old seedlings; the hypocotyls were from 2 to 4^{cm} long.
3. A mixture of the hypocotyls and plumules from six-day old etiolated seedlings.
4. The roots, stems, and buds of seven-day old seedlings grown in the light were tested separately; the roots were copiously branched and the buds were just opening from between the cotyledons.
5. Whole young plants, exclusive of cotyledons, of ten-day old etiolated seedlings.
6. The leaves and buds, and the roots and stems, of eleven-day old seedlings grown in the greenhouse.
7. The leaves and buds, stems, and roots of thirteen-day old etiolated seedlings.
8. The same tissues of fourteen-day old non-etiolated plants.
9. The leaves and the stems of plants twenty-two days old.
10. The leaves and buds, the upper parts of the stems, the stems from the root crown to a centimeter above the scars of the cotyledons.
11. The roots, stems, and leaves of plants just coming into bloom.
12. The developing seeds and pods.

In every case a good tryptophan reaction was obtained when the tissues were allowed to autolyze in the presence of Witte peptone. In no case, with the questionable exception noted below, could evidence of the presence of an enzyme capable of attacking native proteids be obtained. The one exception was furnished by the tests conducted on young hypocotyls in which edestin was added to the mixture of minced tissues and water. In this case a very faint tryptophan reaction was obtained, sufficient to raise the question of the existence of a tryptase in these tissues. Accordingly the following experiment was carried out. Ten grams of minced hypocotyls (1 to 3^{cm} long) from four-day old bean seedlings were placed in each of two flasks and 25^{cc} of water added. One flask was heated for several minutes in the steam sterilizer and then cooled. To each flask was added 0.25^{cm} of phaseolin, 0.25^{cm} of phaselin, and 1^{cc} of toluol. The corked flasks were kept in the incubator for three days. After

the digestion the contents of each flask were strained through cotton gauze, and 20^{cc} of each fluid precipitated with an equal volume of tannic acid reagent. Duplicates of 15^{cc} each from both filtrates were analyzed for nitrogen.

Nitrogen in analyses of filtrates of unboiled digestion = 0.0036^{gm}
Nitrogen in analyses of filtrates of boiled digestion = 0.0035^{gm}

It is therefore evident that no enzyme capable of acting on the native proteids of the bean is present in the young hypocotyls.

COMPARATIVE CONTENT OF EREPTASE IN VARIOUS TISSUES OF *PHASEOLUS VULGARIS*

In making any study of the relative quantities of ereptase in different tissues it is necessary to have some basis of comparison. Manifestly the dry weight of vegetable tissues is not a very satisfactory standard, since in many cases the cell walls constitute the greater part of the dry weight. The ideal way would be to use the weight of protoplasm as a basis for comparison, but this is out of the question. The best substitute seemed to be the quantities of the nitrogen in the tissues. The first measurements of the amount of ereptic activity in the tissues were carried out in the following manner. Nitrogen determinations were made on the fresh tissues to be tested, and then portions of each tissue, of such a weight that each portion contained 0.02^{gm} of nitrogen, were weighed out into small tared flasks. Into each flask 10^{cc} of a 10 per cent. Witte peptone solution were measured and the total weight of the digestion made up to 25^{gm} by the addition of distilled water. After the addition of 1^{cc} of toluol to each digestion the flasks were tightly corked and kept in the incubator for twenty-four hours at 41° C., the contents of each flask being shaken twice during that period. At the close of the digestion each mixture was strained through dry cotton gauze, and 20^{cc} of each fluid mixed with 20^{cc} of tannic acid reagent. The precipitates were filtered off on dry filters and the filtrates analyzed for nitrogen in duplicates of 15^{cc} each. Minor changes were made in this procedure in the subsequent series of determination, so that the results obtained thereby were not strictly comparable with the first series; since it was impossible to test the same tissues again the results of the first, and less satisfactory series, are nevertheless given:

Tissue	Percentage of N	Weight of tissue used	N in 15cc of filtrate from tannic acid precipitate
Leaves of mature plants.....	0.68	2.941gm	0.0204gm
Developing pods.....	0.39	5.130 "	0.0240 "
Developing seeds.....	0.95	2.105 "	0.0283 "

From such results as these it is impossible to tell how much of the nitrogen found in the filtrates from the tannic acid precipitation is due to products of the digestion of Witte peptone and how much is non-proteid nitrogen present in the tissues used. Moreover, Witte peptone is not completely precipitable by tannic acid, certain of the very soluble peptones not being thrown down by that reagent. In the remaining estimations of comparative ereptic power two digestions were made with each tissue, one a boiled control. As before, the quantity of tissue taken was that which should contain 0.02gm of nitrogen. The digestion mixtures were made up to 30gm instead of 25gm, as this quantity was found to be more convenient. Moisture determinations were also made on each tissue so that comparisons of the dry weights could be made. The results of this study are given in the accompanying table.

The table shows that although the method is not absolutely exact, yet fairly good duplicates are obtained in different digestions with the same tissue. The digestion in a number of cases was vigorous, noticeably so with the roots where more than half of the Witte peptone in the digestion mixture was hydrolyzed. It should be said that the roots bore a few small tubercles which were removed so far as possible, yet the minute ones remaining may have had some influence on the results. It is suggestive to note that it is not the seeds and cotyledons which contain the most enzyme per unit of nitrogen, but rather it is those tissues where active metabolism is occurring. It is unfortunate that we are unable to use the weight of protoplasm as a basis for comparison. The use of the nitrogen content as a standard is well enough between the various active tissues, but when these are compared with tissues gorged with storage proteids it is evident that the storage tissue contains far less protoplasm to a given weight of nitrogen. The error which the presence of stored proteids introduces into our comparative results is evident in the case

Tissue used	Percentage of nitrogen	Weight of fresh tissue	Dry weight of the tissue used	N in 15cc of filtrate from tannic acid ppt. unboiled	Calculated amount for whole unboiled digestion	N in 15cc of filtrate from tannic acid ppt. boiled	Calculated amount for whole boiled digestion	Nitrogen converted by the digestion	Witte peptone corresponding to nitrogen converted[1]
Ungerminated seeds I	3.80	0.526gm	0.409gm	0.0141gm	0.0564gm	0.0108gm	0.0432gm	0.0132gm	0.0911gm
Cotyledons of 5-day seedlings	1.74	1.149	0.455	0.0156	0.0624	0.0117	0.0468	0.0156	0.1076
Cotyledons of 10-day seedlings	0.72	2.777	0.653	0.0213	0.0852	0.0120	0.0480	0.0372	0.2567
Ungerminated seeds II	3.80	0.526	0.409	0.0141	0.0564	0.0111	0.0444	0.0120	0.0828
Cotyledons of 3-day seedlings	1.71	1.170	0.404	0.0144	0.0576	0.0102	0.0408	0.0168	0.1159
Hypocotyls of 3-day seedlings	0.62	3.226	0.286	0.0174	0.0696	0.0114	0.0456	0.0240	0.1656
Roots—blossoming stage	0.31	6.452	0.807	0.0321	0.1284	0.0123	0.0402	0.0792	0.5465
Leaves—blossoming stage	0.72	2.777	0.404	0.0199	0.0796	0.0096	0.0384	0.0412	0.2843
Stems—blossoming stage	0.23	8.696	1.304	0.0240	0.0960	0.0126	0.0504	0.0456	0.3146
Ungerminated seeds III	3.80	5.26	.499	0.0138	0.0552	0.0111	0.0444	0.0108	0.0745
Stems—plants mixed age	0.23	8.696	1.304	0.0240	0.0960	0.0132	0.0528	0.0432	0.2981

[1] The Witte peptone used in these experiments was found to contain 14.5 per cent. of nitrogen; the figures in this column were accordingly obtained by multiplying the values of the preceding column by 6.9.

of the emptying cotyledons. The cotyledons of ten-day old seedlings contain much more ereptase than those of the three and five-day old ones. The ten-day cotyledons were green, shriveled, and probably contained no proteids except those of the protoplasm. As a consequence, the number of cotyledons needed to contain 0.02^{gm} of nitrogen was far larger, the number of cells used much greater, and the amount of protoplasm probably far in excess of the amount in the digestion with younger cotyledons and ungerminated seeds. It is doubtful if there is any increase of ereptase per cell during the process of germination; if that is the case, the amount of ereptase per unit of protoplasm contained in the seeds and young cotyledons is much more clearly indicated by the results obtained with the exhausted cotyledons than by those obtained with the storage tissues themselves. In many cases it is evident that there is no special quantity of ereptase formed in the germinating seeds for the purpose of digesting the reserve proteids. The unexpected activity of the root tissue is probably connected with some processes, as yet unknown, which occur there and which are associated with the nitrogenous metabolism of those organs. VERNON obtained just such a suggestive result with the kidneys of several warm blooded animals; it is probable that there are processes occurring in that organ which are now unknown, but which involve a rapid nitrogenous metabolism.

CONCLUSIONS.

The results of this study have shown that, as was to be expected, the proteids of the seeds of *Phaseolus vulgaris* undergo proteolysis during germination as a preliminary to the transportation of the nitrogen and its utilization in the formation of new organs. There are three ways in which this process might be carried out: by the action of a tryptic enzyme; by the combined action of the protoplasm and of an enzyme which is, by itself, incapable of carrying out the whole process; or by the action of the protoplasm alone. The results of a study of the proteolytic enzymes of the resting and germinating bean show that there is no enzyme present which is able to digest the proteids of the seed. There is present, however, an enzyme of the ereptase group which is capable of digesting the proteoses resulting from the partial hydrolysis of the seed proteids. It may be,

therefore, that the protoplasm of the cells starts the process of proteid decomposition, and carries it to some stage at which the ereptase takes up the work and completes the process. That the protoplasm has some hand in the process is made evident by the fact that killing the protoplasm is sufficient to stop the proteolysis, although the means taken are those which do not inhibit the action of enzymes. Moreover, when the cells of the cotyledons are placed under abnormal conditions, the hydrolysis of the proteids ceases. It is, of course, possible that the complete hydrolysis is carried out by the protoplasm and that the ereptase has no share in it. There is something to be said for either view. It might be pointed out that the ereptic activity as shown by the tissues of the cotyledons when acting on Witte peptone is quite sufficient to accomplish the ereptic part of the complete proteolysis. Moreover, the very fact that an enzyme is present which is capable of doing part of the work of cleavage would lead one to infer that it had some part to play in the process. On the other hand, it has been shown that the cotyledons contain relatively less ereptase than the ordinary vegetative organs, and that there is no increase, or at the most a very slight one, in the enzyme content during germination.

The investigation of the other ograns of the plant shows that they all contain the ereptase in somewhat varying quantities, the roots containing the most per unit of nitrogen. There is no evident explanation for these quantitative variations, nor for the function of the enzyme in the metabolism of the cells.

We have good reasons for believing that the life of every active cell is intimately bound up with a round of chemical changes of which one part consists in a cleavage of the protoplasmic proteids. It is quite conceivable that the means by which the cleavage is effected may vary in different cells; in some the whole process may be carried out by the protoplasm itself, unassisted by any of its enzymatic tools; in other cells the protoplasm may start the process and split off proteoses from itself which are subsequently attacked by an ereptic enzyme and broken down to the amido-acids, hexon bases etc.; in still other cases it may be that the complete hydrolysis is carried out by an enzyme or combination of enzymes. The bridging of the gap between widely different forms by a series of small variations

is a characteristic of the morphology of living organisms. That same sort of bridging is no less characteristic of their physiological processes. For example, we find all stages of dependence upon oxygen, from plants which are killed by its absence to those which are killed by its presence. It is not inconceivable that there exists an analogous range of differences in the means which various plants take to accomplish the cleavage of their metabolic proteids.

At any rate it has been shown by the researches of various investigators that enzymes are present in animal and vegetable tissues of both the tryptic and ereptic type. There has not yet been found a tissue which has been definitely shown to contain no proteolytic enzymes whatever, and which must therefore complete its processes of proteolysis by the activity of its protoplasm. Our knowledge is too limited to allow us to say that such tissues do not exist; that point must be left for future investigations.

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LABORATORY OF PLANT PHYSIOLOGY,
SHEFFIELD SCIENTIFIC SCHOOL,
YALE UNIVERSITY.

CONTRIBUTIONS TO THE BIOLOGY OF RHIZOBIA. IV: TWO COAST RHIZOBIA OF VANCOUVER ISLAND, B. C.[1]

ALBERT SCHNEIDER.

(WITH THREE FIGURES)

LEGUMINOUS plants are comparatively rare on Vancouver Island. Two species, beach vetch (*Lathyrus maritimus* Bigel.) and beach clover (*Trifolium heterodon* Gray), were more carefully examined with regard to root nodule formation and proved rather interesting. Of these two plants, the vetch is by far the more common and more widely distributed. It is creeping, climbing, and spreading in habit, thus being endowed with certain advantages in the struggle for existence. It climbs upon and spreads over the smaller and less fortunate herbs, thus gaining access to the desirable air and sunlight. The beach clover, in common with the majority of clovers, was originally adapted to the sunlit open ground, but the tree vegetation of the island has compelled it to occupy an extreme shore position. In the struggle for existence it has evolved into a hardy persistent plant, clinging tenaciously to the scant soil in the crevices of rocky shore slopes, approaching the high tide mark. During the often prolonged heavy inland winds of the winter months, these plants are thoroughly drenched by the salt waves and salt spray without suffering any inconvenience whatever. In appearance beach clover is not unlike our familiar white clover (*T. repens*); the plants however are larger, and the flowers are larger and more showy.

The roots of both plants were well supplied with rhizobia-bearing nodules. Sections were made of these and examined microscopically. In the case of beach clover the rhizobia presented the general morphological characteristics of those found in the nodules of red and white clover (*fig. 1*). That is, they were of the very characteristic Indian club form, with very distinct bodies described by some as granules

[1] The work here recorded was done at the Minnesota Seaside Station, session of 1904.

of amylodextrin or degenerate proteids, and which I have elsewhere designated as sporoids. The etiology and function of these bodies still remains to be determined. Some of the rhizobia showed distinct traces of forking (Y-forms), but the majority were of the Indian club form, derived from Y-forms, while a few were of uniform width, evidently derived from simple unbranched rod forms.

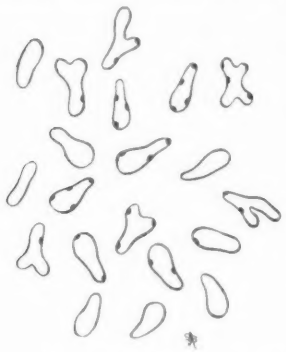


FIG. 1.—Rhizobia from the root nodules of beach clover (*Trifolium heterodon* Gray), showing extreme form variation of *R. mutabile*, due to hyper-nutrition; same organisms with the so-called sporoids.

The rhizobia of beach vetch were rather remarkable for their branching (*fig. 2*). They present the general morphological characteristics of the rhizobia of sweet clover, bur clover, and other vetches. The branching, however, is more pronounced than in any other form of this type hitherto examined. The branching is dichotomous and may be either unipolar or bipolar. The highly refractive sporoids are not present, nor have they ever been observed in rhizobia of this type.

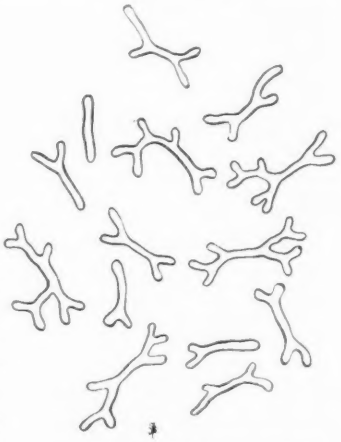


FIG. 2.—Rhizobia from the root nodules of beach vetch (*Lathyrus maritimus* Bigel.), representing the extreme branching form of *R. mutabile*, due to hyper-growth.

It is highly probable that these two rhizobia (of beach vetch and beach clover) represent two extreme natural form types of *Rhizobium mutabile* (*R. leguminosarum* Frank). Assuming that the rod forms and simple Y-forms are the original normal types, we have in the rhizobia of beach clover (and in other clovers) the extreme form deviation, apparently due to hyper-nutrition; and in the rhizobia of

beach vetch the extreme branching form, due to hyper-growth. This supposition is strengthened by the study of rhizobia in artificial culture media. Grown in the same culture media the rhizobia of sweet clover and red clover are morphologically identical. Whether they are physiologically identical has not been determined, although this is also probable according to the inoculation experiments of NOBBE, HILTNER, and HOTTES, of Germany, which are supported by the research of MOORE of the Department of Agriculture. It is quite apparent that these variations in form are due to the food-supply or nutritional changes, variations in the supply of oxygen and moisture, variations in chemical reaction, temperature, light, and other ecological factors. It is possible by means of special culture media to augment very decidedly the branching in the rhizobia of sweet clover and to induce other morphological variations, as has frequently come under my observation. It should be noted that the so-called sporoids do not appear in the rhizobia in artificial culture media. This is of considerable importance, as it was once believed that these bodies were of sufficient significance to be of specific rank. It would appear from these observations that they are more likely by-products stored within the cell, having perhaps food value, derived from the host plant. If this supposition is correct, the theory that they are etiologically sporoidal in nature is untenable.

It seems very probable, and wholly within the range of the possible, that the two extreme form types here described are phylogenetically derived from an original form type similar to, but not necessarily identical with, the form found in *Cassia Chamaecrista*, *Robinia pseudacacia*, *Trigonella foenum graecum*, and *Amphicarpaea comosa*, which were formerly described as distinct species. From this it does not follow, of course, that these form types are of necessity variations of one and the same species, although the evidence thus far deduced points in that direction.

The presence of the *Infectionsfäden* was noted (*fig. 3*), and nothing new regarding their significance was discovered, only I wish to state, by way of readjustment of what was stated in previous papers, that in all probability these threads are merely a phenomenon dependent upon the infection of the root cells by the rhizobium. The action of the motile forms in the apical area of the root nodule (formerly

described as a distinct species, *R. Frankii*) causes the appearance of the threads in a manner already explained.

The question of Rhizobia species is not yet settled, and cannot be settled until our information regarding their biology is much more complete. The fact that extensive research work has already been done by a large number of investigators without coming to any conclusion regarding species should serve as a very suggestive lesson

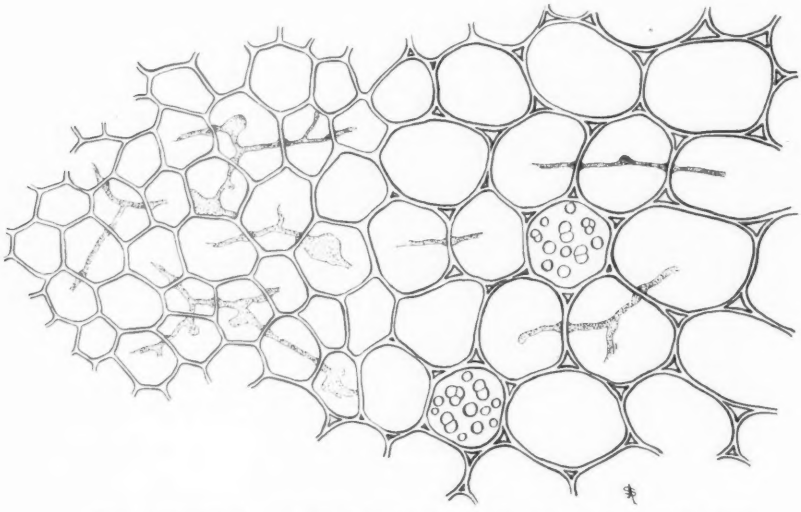


FIG. 3.—Portion of longitudinal section of root nodule of beach vetch (*Lathyrus maritimus* Bigel.) showing *Injectionsfäden*, more abundant in the apical area; rhizobia are not shown; two starch-bearing cells.

to species hunters. In the case of *R. mutabile*, the question is now arising as to whether it is a microbe (Schizomycete) or a hyphal fungus, a question which we hope to discuss more fully in some future paper. All investigators are agreed that *R. mutabile* is an organism showing extreme polymorphism. It would appear to be an organism wonderfully adapted to test the De Vriesian theory of mutation as it applies to low organisms. At this time the only statement ventured is that apparently constant natural variations in *R. mutabile*, as above indicated, at once become transformed into "variable or unstable vari-

ability" in artificial culture media. It would appear that the newer conception of species as based upon the facts of ecology; study of mutation, constant and variable; crossing, artificial and natural, etc., will necessitate a complete change in our present systems of classification.

California College of Pharmacy,
San Francisco

BRIEFER ARTICLES.

THE VITALITY OF SEEDS.

In the autumn of 1879 I began the following experiments, with the view of learning something more in regard to the length of time the seeds of some of our most common plants would remain dormant in the soil and yet germinate when exposed to favorable conditions. I selected fifty freshly grown seeds from each of twenty-three different kinds of plants. Twenty such lots were prepared with the view of testing them at different times in the future. Each lot or set of seeds was well mixed in moderately moist sand, just as it was taken from three feet below the surface, where the land had never been plowed. The seeds of each set were well mixed with the sand and placed in a pint bottle, the bottle being filled and left uncorked, and placed with the mouth slanting downward so that water could not accumulate about the seeds. These bottles were buried on a sandy knoll in a row running east and west, and placed fifteen paces northwest from the west end of the big stone set up by the class of 1873. A bowlder stone barely even with the surface soil was set at each end of the row of bottles, which were buried about twenty inches below the surface of the ground. I should make an exception in the case of the acorns, which were placed in the soil near the bottles, and not inside bottles. At the end of five, ten, fifteen, twenty, and now twenty-five years, sets of these seeds were tested for vitality. The names given in the following table were those in use when the seeds were buried. Some of those marked *x* germinated; none of those marked *o* germinated.

In all the species in the five tests made, eight out of twenty-two failed to germinate; and of the remaining fourteen, some of ten species germinated, often when they had been buried twenty-five years. The acorns buried near the bottles were all dead at the end of two years. I soon began other experiments with acorns, and in addition planted some black walnuts with the acorns. On a sandy knoll these nuts were buried at various depths in a hole the depth of which was equal to the length of a spade and handle, some of them three feet or more below the surface. After they had remained nearly two years, some of them were examined with the following results: Some of the walnuts and acorns planted only a few inches beneath the surface had come up the next summer after planting, while those planted at

Names of seeds tested	5th year	10th year	15th year	20th year	25th year
Amaranthus retroflexus	x	x	x	x	x
Ambrosia artemisiaefolia	o	o	o	o	o
Brassica nigra	o	x	x	x	x
Bromus secalinus	o	o	o	o	o
Capsella Bursa-pastoris	x	o	x	x	x
Erechthites hieracifolia	o	o	o	o	o
Euphorbia maculata	o	o	o	o	o
Lepidium virginicum	x	x	x	x	x
Lychnis Githago	o	o	o	o	o
Maruta Cotula	x	x	x	o	x
Malva rotundifolia	x	o	o	x	o
Oenothera biennis	x	x	x	x	x
Plantago major	o	o	x	o	o
Polygonum Hydropiper	o	x	x	x	x
Portulaca oleracea	o	x	x	x	x
Quercus rubra	o	o	o	o	o
Rumex crispus	x	?	x	x	x
Setaria glauca	x	x	x	o	x
Stellaria media	x	x	x	x	x
Thuja occidentalis	o	o	o	o	o
Trifolium repens	o	o	o	o	o
Verbascum Thapsus	x	?	x	x	o

a depth of about eight inches to two feet or a little over had all decayed. All the walnuts deeply planted had decayed, but some of the acorns planted two or three feet below the surface were still alive, or rather the young plants were alive. They had probably started soon after planting, as the cotyledons were exhausted, their nourishment having been used in developing roots and pushing up an ascending axis.

On August 12, 1889, after a part of the nuts had been planted and undisturbed for two months less than four years, I examined them. Eight acorns were found alive, with the roots about like the roots of those dug up two years before. The ascending axis in most cases was slender and crooked, with a delicate white apex. In one case there was no ascending axis, but a solid, fleshy root, apparently alive.

In all tests of the seeds buried in bottles, the results have been indefinite and far from satisfactory. I mean by this that I have never felt certain that I had induced all the sound seeds to germinate. I moisten the sand containing the seeds, and forthwith a goodly number germinate, and then they come slowly straggling along. I dry the soil and wait a few days, and after moistening, in a few days more seeds germinate. Why was I unable to induce them to start when treated to various degrees of temperature and moisture for seven months?

We see this important point. It is to the advantage of the plants not to shoot up all of their seeds at one time, but to retain a good portion alive

in the soil to be ready for stocking the earth in successive years. Again, we must consider that it makes very little difference whether all the seeds live over for a time or only a small proportion of those which were produced, as a living seed now and then left is enough to save the stock and produce new crops of seeds.

The seeds I began testing in August 1894 were kept in trial until November of that year, when the plates containing the dry sand were set away dry until the next spring, and kept in test for that year until November 1895. In this second year some seeds of eight species germinated.

In the sets of seeds which were put in condition, as I supposed, to germinate in July 1899, after being buried twenty years, some seeds of eighteen species grew during the following four months, when the plates were set away till the next April (1900), at which time the sand was occasionally wet. During this period, some seeds of mustard, mallow, shepherd's purse, and chickweed germinated.

In September 1882 I selected of the second crop of red clover five plants within a few feet of each other, which seemed much alike. The seeds of fifty good heads of each, containing 1260–1820 seeds, were shelled, and ever since, till tested, they have been kept, each lot by itself, in a two-ounce bottle well corked. For a portion of the time they were exposed to the light; for some years they have been kept in a dark closet. Nearly twelve years after collection, fifty seeds of each lot were tested for vitality, with the following results:

Of no. 1	24	germinated
Of no. 2	8	"
Of no. 3	34	"
Of no. 4	25	"
Of no. 5	0	"

Two weeks later another test of fifty seeds each was made:

Of no. 1	31	germinated
Of no. 2	10	"
Of no. 3	32	"
Of no. 4	21	"
Of no. 5	4	"

This is an average for both tests of 35.8 per cent. The difference in germination percentage of these lots of seeds perhaps may be accounted for by the presence of weevil in a few seeds; by difference in the size of seeds, or the stage of maturity; by individual peculiarities of the different plants. By some means, since testing, the bottle no. 4, containing the seeds, has been lost.

On November 16, 1904, over twenty-two years from collecting, I began tests of 100 seeds of numbers 1, 2, 3, 5, with the following results:

Of no. 1	0	germinated
Of no. 2	0	"
Of no. 3	5	"
Of no. 5	1	" possibly a second one

—W. J. BEAL, *Agricultural College, Michigan.*

SOME MEXICAN SPECIES OF CRACCA, PAROSELA, AND MEIBOMIA.

(WITH PLATE V)

THE genus name Cracca of Linnaeus (1753) has of late years been restored in place of the Tephrosia of PERSOON (1807), which is clearly a synonym, as has been well pointed out by Mr. E. G. BAKER.[1]

Dalea, although first proposed by LINNAEUS in 1737, was reduced by him in 1753 to Psoralea. It was not restored until after PATRICK BROWNE in 1756 had published his Dalea, and therefore the next available name, Parosela, must be used.

The Meibomia of ADANSON (1763) has properly been taken up in place of Desmodium (DESVAUX, 1813), which must be treated as a synonym, though there is some ground for regarding the two names as representing different genera.

An examination of these three genera by Mr. ROSE in connection with his *Studies of Mexican plants* has shown that they are greatly in need of revision, and considerable work has been done with a view to meeting this want. Mr. PAINTER has a revision of the Mexican and Central American species of Meibomia well advanced. It was not the intention to publish any notes on these genera until our revisions were completed; but there has been considerable demand, both from general collectors and from botanists who have been working on Mexican fungi, for correct names for certain species; and we have concluded to publish a few of the new combinations and new species at the present time.

Cracca talpa (S. Wats.) Rose.—*Tephrosia talpa* S. Wats. Proc. Am. Acad. **22**:405. 1887.

Cracca macrantha (Rob. & Greenm.) Rose.—*Tephrosia macrantha* Rob. & Greenm. Proc. Am. Acad. **29**:383. 1894.

Cracca Pringlei Rose, sp. nov.—Herbaceous perennial much branched at base; branches 10 to 20^{cm} long, appressed-pubescent: leaflets 7 to 10

[1] Jour. Botany, Jan. 1900.

pairs, oblong, 8 to 15^{mm} long, green above and with scattered hairs, densely cinereous beneath, rounded and mucronate at tip: inflorescence short and compact, not much exceeding the leaves: calyx lobes narrow: corolla purplish, the banner 15^{mm} long: ovary very hairy; pods (immature) 4^{cm} long.

Collected by C. G. Pringle on hills of Las Sedas, Oaxaca, July 22, 1897 (no. 6741).

Type in the U. S. National Herbarium.

Parosela mutabilis (Cav.) Rose.—*Psoralea mutabilis* Cav. Ic. **4**:65. *pl. 394.* 1797. *Dalea mutabilis* Willd. Sp. Pl. **3**:1339. 1801.

Parosela acutifolia (DC.) Rose.—*Dalea acutifolia* DC. Prod. **2**:245. 1825.

Parosela uncifera (Schlecht. & Cham.) Rose.—*Dalea uncifera* Schlecht. & Cham. Linnaea **5**:580. 1830.

Parosela triphylla (Pavon) Rose.—*Dalea triphylla* Pavon, Linnaea **12**:289. 1838.

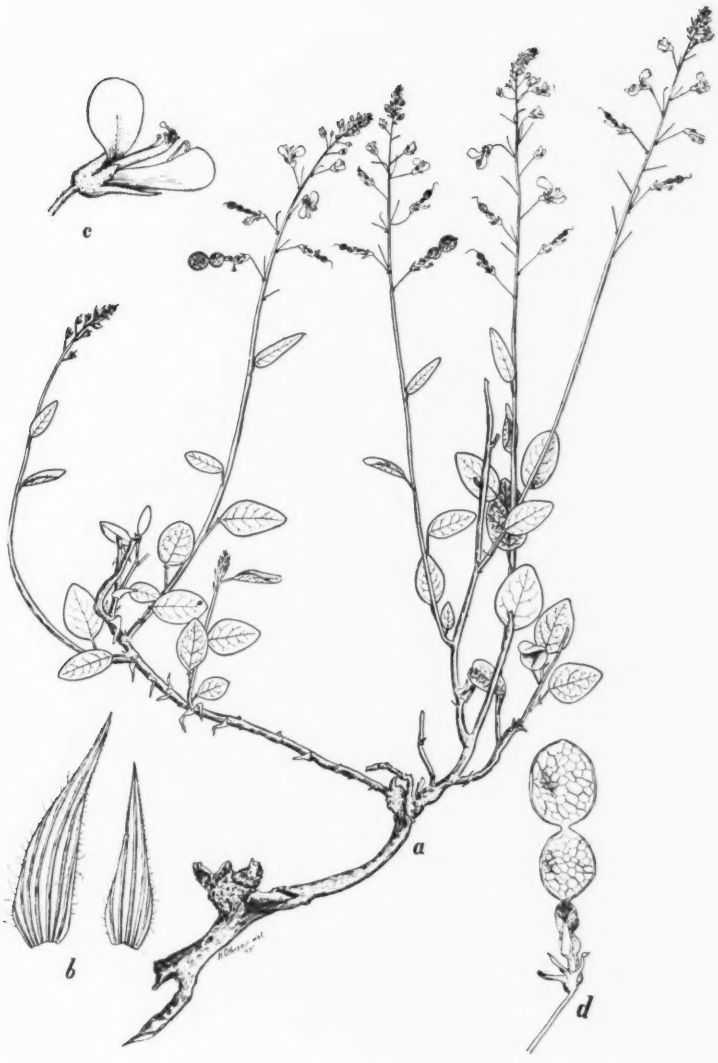
Parosela procumbens (DC.) Rose.—*Dalea procumbens* DC. Prod. **2**: 246. 1825.

Meibomia (HETEROLOMA) **Metcalfii** Rose & Painter, sp. nov.—Herbaceous, erect with ascending, striate branches: leaves trifoliolate, narrowly ovate-lanceolate, 3 to 5^{cm} long, 0.6 to 1.5^{cm} wide, obtuse at base, acute at apex, margins revolute, upper surface sparsely hirsute with short hairs, dark green, under surface lighter green and glabrous, veins more prominent below than above;. petioles angled, of lower leaves 3^{cm} long, of upper leaves nearly wanting; stipules deciduous: inflorescence in open panicles, its racemes terminal and lateral: flowers small, on pubescent pedicels; bracts acuminate, pubescent, early deciduous: calyx purplish, of 5 unequal teeth: corolla small, purplish: ovary pubescent, stipitate; loment decidedly stalked, of 2 to 5 joints, these much longer than broad and covered with uncinate hairs.

Collected by Mr. O. B. Metcalf in the Black Range, Animas Creek, Grant County, New Mexico, on ditch banks, altitude 1,500^{m}, July 13, 1904 (no. 1137).

A species with the aspect of *M. paniculata* (L.) Kuntze, but more closely related to *M. Lindheimeri* Vail.

Meibomia (HETEROLOMA) **pinetorum** Rose & Painter, sp. nov.—Herbaceous, stem glabrous, trailing, 1 to 2^{m} long, with ascending branches: leaves trifoliolate; leaflets 1 to 3.5^{cm} long, 1 to 3^{cm} wide, thin, broadly oval, obtuse and mucronate at apex, very sparingly hirsute on the upper surface with scattered appressed hairs, the lower surface lighter green with the primary veins prominent, covered with fewer scattered appressed



MEIBOMIA PALLIDA ROSE and PAINTER

hairs; petioles 1 to 3^{cm} long, glabrous; petiolules of lateral leaflets 1 to 3^{mm} long, of terminal leaflets 8 to 15^{mm} long, pubescent; stipules persistent, small, long-acuminate, glabrous: inflorescence in a simple terminal or lateral axillary raceme; flowers purple, on filiform pedicels; floral bracts deciduous, ovate, acuminate, somewhat puberulent: calyx unequally 5-toothed, pubescent: loment 3 to 4, rarely 5-jointed, almost sessile, the joints covered with uncinate pubescence.

Collected by Mr. C. G. Pringle about Trinidad Iron Works, Hidalgo, Mexico, in pine woods, altitude 1650^{m}, September 15, 1904 (no. 8890).

Type in U. S. National Herbarium, no. 461381.

This species is nearest *M. orizabana* (Hemsl.) Kuntze, but is easily distinguished from that species by its obtuse, oval leaves, prostrate habit, and loment more deeply constricted above.

Meibomia xylopodia (Greenman) Rose & Painter.—*Desmodium xylopodium* Greenman, Proc. Am. Acad. **39**:80. 1903.

Meibomia (CHALARIUM) **pallida** Rose & Painter, sp. nov.—Low, shrubby, 20 to 30^{cm} high: leaves all unifoliolate, the lowest orbicular or broadly oval, obtuse, upper oblong, mucronulate, all pale green, obtuse at base, above covered with short uncinate pubescence, less so and reticulate-veined beneath, midrib prominent, pubescent; petioles pubescent, 2 to 5^{mm} long; stipules deciduous, ovate-acuminate, pubescent and with prominent veins; stipels subulate, pubescent, 1^{mm} or less long: inflorescence in a terminal simple raceme which is densely pubescent; flowers purple; pedicels when fully matured 5 to 6^{mm} long, pubescent; bracts deciduous, lanceolate-ovate, long-acuminate, ribbed: calyx pubescent, the teeth unequal: ovary appearing spirally twisted, pubescent; loment about 5-jointed, the joints inflated, glabrous, reticulate-veined.

Collected by Mr. E. W. Nelson at Huilotepec, Oaxaca, Mexico, altitude 25^{m}, May 4 to 11, 1895 (no. 2587).

Type in U. S. National Herbarium, no. 40034.

The affinity of this species is with *M. xylopodia* (Greenman) Rose & Painter, but it differs in the glabrous loments and the paler green leaves.

EXPLANATION OF PLATE V: *a*, plant (×⅔); *b*, floral bracts (×7); *c*, flower (×5); *d*, fruit (×2½).

Meibomia (CHALARIUM) **rubricaulis** Rose & Painter, sp. nov.—Low shrub, twigs reddish-brown, older ones with striate and whitish bark: leaves small, trifoliolate, petiolate; petioles 5 to 12^{mm} long, covered with short pilose hairs; terminal leaflets obovate, 8 to 12^{mm} long, 6 to 10^{mm} broad, the lateral leaflets mostly oval, at times somewhat obovate, rounded, mucronulate at apex and rounded at base, upper surface minutely

roughened with very short hairs, beneath pilose, reticulate, the veins more prominent beneath than above; stipules long-persistent, subulate lanceolate: inflorescence terminal and lateral, of simple racemes; flowers purple, on pilose pedicels (5^{mm} long); bracts lanceolate, early deciduous: calyx lobes equal, obtuse, pubescent: ovary puberulent; loment 2 to 5-jointed, decidedly stipitate, the joints reticulate-veined, 4^{mm} long and 3^{mm} wide, with a very narrow isthmus (not quite central) rarely half as long as adjacent joints.

Collected by Dr. E. Palmer at Tequila, Jalisco, August-September 1886 (no. 398); by C. G. Pringle, on rocky hillsides near Guadalajara, Jalisco, October 1, 1891 (no. 3877); by J. N. Rose & Jos. H. Painter, vicinity of Rio Blanco, Jalisco, September 30, 1903 (no. 7492); and by C. G. Pringle on mountains about Etzatlan, Jalisco, October 2, 1903 (no. 11413; type, no. 460875 of the U. S. National Herbarium).—J. N. ROSE and JOS. H. PAINTER, *U. S. National Museum*.

A NEW KRYNITZKIA

MR. W. N. SUKSDORF, who for a number of years has made quite extensive collections of plants in the northwest, more especially in the state of Washington, sent recently to the Gray Herbarium a consignment of specimens containing several species of particular interest. Among these rarities is a Krynitzkia which deserves an early record. Flowering specimens of this plant were first secured in the spring of 1901; and in June of the past season fruiting material was obtained. These collections have been placed at the disposal of the writer for study, and a careful comparison with the entire representation of the genus in the Gray Herbarium shows that the Suksdorf plant is most nearly related to *K. oxycarya* Benth. and *K. rostellata* Greene. From type material of both these species the Suksdorf plant differs in several important characters, and it seems best, therefore, to regard it as specifically distinct. The writer takes pleasure in dedicating the new species to its collector. The plant may be characterized as follows:

Krynitzkia Suksdorfii Greenman, n. sp.—A small annual: stem erect, 0.5 to 1.5^{dm} high, simple or branched from the base, canous-strigose: leaves opposite below, alternate above, spatulate to linear, 0.5 to 1.5^{cm} long, 1 to 3.5^{mm} broad, obtuse, entire, subappressed-tuberculate-hispid: flowers small, sessile: calyx deeply 5-parted, about 2.5^{mm} long in anthesis, pubescent with spreading slightly curved stiff hairs, persistent, becoming 4^{mm} long in fruit and surrounding the single mature nutlet; lobes of the calyx linear, acute: corolla about 3^{mm} in length; lobes subrotund, 1^{mm} in

diameter: stamens adnate to the corolla-tube for about one-half its length; anthers sessile: mature nutlets narrowly ovate, 2.5^{mm} long, acuminate, smooth and shining, pale chocolate-colored, somewhat mottled with darker spots; the ventral surface slightly flattened and the groove bifurcated at the base.—WASHINGTON: on dry hillsides near Rockland, Klickitat County, 18 April 1901, *Suksdorf*, no. 1495 (flowering specimen), and 8 June 1904 (fruiting specimen); on dry hillsides near Dallas City, 17 April 1901, *Suksdorf*, no. 2346 (flowering specimen). Type in herb. Gray.

This species differs from *K. oxycarya* Benth. in having shorter broader leaves, somewhat larger corolla, more prominently beaked, darker colored and mottled nutlets which are bifurcately grooved on the ventral side near the base. From the Californian *K. rostellata* Greene, *K. Suksdorfii* differs in having somewhat smaller habit, shorter, broader leaves, much shorter branches of the inflorescence, slightly larger corolla with broader corolla-lobes, and shorter calyx-lobes in the fruiting state.—J. M. GREENMAN, *Gray Herbarium*.

CURRENT LITERATURE.

BOOK REVIEWS.

The origin of species and varieties by mutation.

PROFESSOR DEVRIES[1] has hit upon a method for presenting his experiments and theories to the English-reading public that is as happy as it is unique. It is commonly the fate of an epoch-making work, such as *Die Mutationstheorie* has proved itself to be, to undergo a translation into other tongues in the course of three or four years, without alteration except for some inevitable changes for the worse. DeVries has taken into his own hands the preparation of the English exposition of mutation, and we have as a result a book that is written for a very different audience, couched in different language, and prepared in the light of the experiments and discussions of the past four years. The investigator who desires the minutiae of DEVRIES' experimental results will still have recourse for the most part to the earlier volumes, but as investigators are supposed to be conversant with the German language and with the technical terminology there employed, no difficulty results. The investigator, however, will require the present volume for the broader viewpoint, and for the contributions that have appeared since 1901. The great and undisputed field for the present volume is the presentation of mutation to the large and important audience of intelligent people to whom German is a foreign language, and technical terminology more so.

The volume under consideration is based on a course of lectures given at the University of California in the summer of 1904, and was edited by D. T. MACDOUGAL of the New York Botanical Garden, whose experiments and contributions have done so much to make Americans conversant with the work of DEVRIES. One of the most valuable and interesting of his lectures is the preliminary one, dealing with theories of evolution and methods of investigation. Here there is an excellent portrayal of the relation that exists between his contributions and those of others; and it is at once clear that the work of DARWIN is not opposed but supplemented and strengthened; DARWIN'S comparative studies resulted in the accumulation of a vast array of material, while DEVRIES' work has systematized this material, and has given us an experimental basis for the belief in evolution. It is to be hoped that the perusal of this volume will put an end to the notion, so widely circulated in the newspapers, that DEVRIES is a destroyer of Darwinism.

[1] DEVRIES, HUGO, Species and varieties; their origin by mutation. Edited by D. T. MACDOUGAL. 8vo., pp. xviii+847. Chicago: The Open Court Publishing Co. 1905.

The following lectures outline the characteristics of elementary species, both in nature and in cultivation, and it is shown that natural selection must play a large part in determining their survival. Varieties are shown to differ from elementary species in not possessing anything that is really new, and in originating commonly by the loss of some quality. Several chapters deal with the various kinds of varieties, retrograde, progressive, and ever-sporting; in the same connection the subject of atavism is elucidated, as well as latent characters, and vicinism or variation under the influence of pollination by neighboring individuals. The lectures on mutations deal not alone with Oenothera, but as well with the peloric toadflax, double flowers, and a great many wild and cultivated plants that are supposed to illustrate mutation. A lecture that will be read with great interest by paleontologists, as well as others, is the one that considers the periodicity of mutations, and the relation that mutation bears to the length of geological time. The final lectures present the topic of fluctuating variations, and perhaps it is here that Darwinians will find least comfort in the work of DEVRIES. The closing words of the book, quoted from ARTHUR HARRIS, will be recognized as most apt: "Natural selection may explain the survival of the fittest, but it cannot explain the arrival of the fittest."

In a review of *Die Mutationstheorie* (BOT. GAZ. **33**:236–239. 1902), it was felt to be too soon to express an opinion concerning the place which that work would occupy in the literature of evolution, although it was the reviewer's intuition that this place would be very high. Of the permanent value of that work, and of the work here under review, there is now no doubt at all. "The greatest contribution since DARWIN" is the universal testimony, and there is a feeling on all sides that the answers to many evolutionary questions are close at hand, and that through the application of experiment. To many of us the new volume brings more than did the old, because we have now seen the author face to face, and have perpetually in mind the modest, lovable man, as well as the renowned investigator.—H. C. COWLES.

MINOR NOTICES.

EMERSON[2] has published the results of experiments in the control of the rust and scab of apples. He finds that the rust of apples due to species of Gymnosporangium can be prevented by spraying with Bordeaux mixture if the first application is made when the gelatinous spore-containing projections first appear on the "cedar apples." This spraying should then be followed by a second spraying about ten days or two weeks after the first. He recommends also that the cedar apples be removed from cedar trees near orchards in the winter or early spring, and that where practicable cedar trees themselves should not be allowed to remain within one mile of apple orchards. The scab he found could be prevented by spraying twice with Bordeaux mixture, once just before the apple blossoms open and again just after the blossoms fall.—E. MEAD WILCOX.

[2] EMERSON, R. A., Apple scab and cedar rust. Bull. Nebraska Exp. Sta. **88**: pp. 21. *figs.* 9. 1905.

CHRISTENSEN[3] has begun the publication of an *Index Filicum*, which is intended to do for ferns what the *Index Kewensis* does for seed-plants. The book will contain a systematic enumeration of the genera, based upon ENGLER and PRANTL'S *Pflanzenfamilien;* an alphabetical enumeration of species and synonyms, which will include all names and combinations of names published from 1753 to 1905 and also names of garden ferns; and an alphabetical catalogue of literature containing critical notes and descriptions of new genera and species. The work will be complete in eleven or twelve parts, and the entire manuscript is ready for printing, awaiting only a sufficient subscription. The first fascicle, just issued, begins the alphabetical list of genera and species, closing with Aspidium.—J. M. C.

MERRILL[4] has attacked the species described in BLANCO'S monumental *Flora de Filipinas*, recognizing that they must be identified and made available so far as possible. He has brought together these identifications in a conveniently arranged bulletin, calling special attention to the species that are yet to be identified. To give some idea of the results attained by this absolutely isolated worker, it may be said that in the two editions of the *Flora* (1837 and 1845) BLANCO described 1127 species and varieties; about 623 of these were proposed as new, and 504 identified with species of other authors, 219 of them correctly and 285 incorrectly. A large proportion of the new species remain unknown, and only 90 are known to be valid.—J. M. C.

HITCHCOCK[5] has published an elaborately illustrated synopsis of the North American species of Agrostis, recognizing twenty-seven species and describing three as new. It is announced as the intention of the department to publish occasional monographs of the larger genera of grasses.—J. M. C.

HUSNOT[6] has published the first part of an illustrated synopsis of the Cyperaceae of France, Switzerland, and Belgium. This part contains Elyna, Kobresia, and Carex. The important characters of each one of the 123 species of Carex are illustrated.—J. M. C.

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[3] CHRISTENSEN, CARL, Index Filicium sive enumeratio omnium generum specierumque Filicum et Hydropteridum ab anno 1753 ad annum 1905 descriptorum adjectis synonymis principalibus, area geographica, etc. Fasciculus 1. pp. 64. Copenhagen: H. Hagerups Boghandel. 1905. Each part 3*s* 6*d*.

[4] MERRILL, ELMER D., A review of the identifications of the species described in Blanco's Flora de Filipinas. Bull. 27, Bureau of Gov't. Labs., Department of Interior. Manila. 1905.

[5] HITCHCOCK, A. S., North American species of Agrostis. Bulletin 68, Bureau of Plant Industry, Department of Agriculture. pp. 68. *pls. 37.* 1905.

[6] HUSNOT, T., Cypéracées: descriptions et figures des Cypéracées de France, Suisse et Belgique. Part 1. pp. 48. *pls. 12.* Cahan, par Athis (Orne): the author. 1905. 5 *fr.*

NOTES FOR STUDENTS.

APOGAMY in the genus Alchemilla has been investigated very thoroughly by STRASBURGER.[7] The work was suggested by MURBECK's researches; and his statements that the embryo of the EUALCHEMILLAE develops from the egg without fertilization, and that there is no reduction of chromosomes in the life history, are confirmed. On the other hand, STRASBURGER reaches a different conclusion as to the origin of the embryo sac of apogamous species of Alchemilla, and has a different theory as to the nature of the embryogeny of these species.

More than forty species were studied. In the European species the pollen, except in a few species, is abnormal, the development being checked at various stages. The pollen mother-cells may disorganize or a tetrad may be formed, but the pollen grains fail to be liberated from the mother-cell. In some cases, the division into tube nucleus and generative nucleus takes place, but such pollen grains disorganize early. There are thirty-two bivalent chromosomes in the pollen mother-cells, and sixty-four univalent chromosomes in the vegetative tissues. In American and African species, an examination of herbarium material showed normal pollen, and it is probable that fertilization occurs in the usual way.

In the ovules of apogamous EUALCHEMILLAE one or more megaspore mother-cells appear. The nucleus passes through the prophases of the heterotypic division up to the synapsis stage, but here the mode of development changes and the nucleus divides by a typical vegetative division. Division in the embryo sac shows the sporophytic number of chromosomes, so that when the egg is formed it contains the vegetative number of chromosomes. When such an egg develops an embryo without fertilization, STRASBURGER regards the phenomenon not as parthenogenesis but as apogamy. Strictly speaking, it would not be even a case of apogamy, but we should have merely an adventitious embryo like one coming from cells of the nucellus. There is not the beginning of a new generation.

The subniveal EUALCHEMILLAE which form normal pollen show a reduction of chromosomes in the formation of the megaspores, and fertilization takes place in the usual way. Those EUALCHEMILLAE which have not lost their sexuality are chalazogams, and some of them form hybrids.

It seems probable that the extraordinary mutation of the EUALCHEMILLAE has weakened the sexuality, and that the failure of fertilization has brought on the apogamous condition.

Rubus and Rosa, which were also examined, have retained their sexuality in spite of extensive polymorphism. The reduction division and fertilization occur regularly.

Dioecism has in many cases given an impulse toward apogamous reproduction, since the separation of male and female individuals decreases the frequency of fertilization.—C. J. CHAMBERLAIN.

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[7] STRASBURGER, E., Die Apogamie der Eualchemillen und allgemeine Geschichtspunkte, die sich aus ihr ergeben. Jahrb. Wiss. Bot. **41**:88–164. *pls. 1–4.* 1905.

UNIVERSITY OF COLORADO

ITEMS OF TAXONOMIC INTEREST are as follows: S. LEM. MOORE (Jour. Botany **43**:137–150. *pl. 471.* 1905), in describing numerous new Australasian species, has described a new genus (*Cratystylis*) of Compositae (Inuloideae), with 3 species, and one (*Nepenthandra*) of Euphorbiaceae (Crotoneae).—H. CHRIST (Bull. Soc. Bot. France IV. **5**:1–69. 1905) has published an account of the Chinese ferns in the collections of the Museum of Natural History, Paris, describing 41 new species and a new genus (*Neocheiropteris*), to replace Cheiropteris, preempted by a genus of fossil plants.—M. A. HOWE (Bull. Torr. Bot. Club **32**:241–252. *pls. 11–15.* 1905) has described new species of Chlorophyceae from Florida and the Bahamas in Halimeda and Siphonocladus, and has established a new genus (*Petrosiphon*) related to the latter.—H. D. HOUSE (*idem* 253–260. *pls. 16–18*), in presenting Viola in New Jersey, recognizes 33 species and describes one as new.—MRS. E. G. BRITTON (*idem* 261–268) has proposed *Pseudocryphaea* and *Dendroalsia* as new genera of mosses, and has described new species in Erpodium.—A. ENGLER (Bot. Jahrb. **36**:213–252. 1905) has described the following new African genera: *Spondianthus* and *Nothospondias* (Anacardiaceae), *Magnistipula* (Rosaceae), *Pretreothamnus* (Pedaliaceae), and *Cycniopsis* (Scrophulariaceae).—M. L. FERNALD (Rhodora **7**:81–92. 1905) has begun the publication of a revision of the North American species of Eriophorum.—J. CARDOT (Rev. Bryol. **32**:45–47. 1905) has published two new genera of acrocarpous antarctic mosses, naming them *Pseudodistichium* and *Skottsbergia*, the peristome of the latter being described as most extraordinary.—A. A. EATON (Fern Bulletin **13**:51–53. 1905) has described a new species and variety of Isoetes from Washington.—J. W. BLANKINSHIP (Montana Agric. Coll. Sci. Studies **1**:35–109. *pls. 1–6.* 1905), in his "Supplement to the flora of Montana," has published new species in Sagittaria, Zygadenus, Salix, Arabis, Physaria, Sedum, Ribes, Saxifraga, Astragalus (2), Lupinus (4), Impatiens, Ammania, Bupleurum, Carum, and Petasites.—JESSIE MILLIKEN (Univ. Calif. Pub. Botany **2**:1–71. *pls. 1–11.* 1904), in a well-illustrated revision of Californian Polemoniaceae, recognizes 6 species of Polemonium, 5 of Collomia, 22 of Navarretia, 36 of Gilia, 31 of Linanthus, and 9 of Phlox, and describes new species in Gilia and Linanthus.—J. M. C.

PEIRCE[8] has studied the dissemination and germination of the seeds of *Arceuthobium occidentale* on the Monterey pine (*Pinus radiata*) of California. The structure and mechanics of the exploding fruit are described in detail; and the seeds were observed in the laboratory to be thrown fifteen feet, sticking to whatever they struck. The so-called seeds, by the way, are seeds enclosed in the inner part of the ovary. The field observations indicate that the majority of seeds strike the leaves of the pine, either of the tree on which they grow or of one near by. In germination the root is negatively phototropic and not very sensitive to contact. When growth is blocked by some obstacle the root forms a thick foot-like holdfast, into which the material in the upper end of the embryo

[8] PEIRCE, GEORGE J., The dissemination and germination of *Arceuthobium occidentale* Eng. Ann. Botany **19**:99–113. *pls. 3–4.* 1905.

is transferred, the seedling becoming mainly a foot. Vascular elements form in the foot, and its central part grows out into the bark. Strands of infecting cells grow toward the medullary rays of the host, through these to the cambium, and finally effect an attachment with the young xylem elements. While the parasite is thus establishing a connection with the young wood, the main part of the haustorium forms a mass of parasitic cells in the cortex of the host. From this cortical mass buds arise and develop into branches that grow out through the bark into the air. The author remarks that "we have here an instance of regeneration without wounding, amputation, or other pathological stimulus. The small part of the seedling which penetrates the host forms and develops stem and leaves; a small part of one organ—the root—develops into a complete plant by forming the missing members."—J. M. C.

STEINBRINCK [9] finds that MEZ made a very imperfect study of the absorption hairs of Tillandsia, and that his erroneous conclusion could have been avoided easily by reference to published investigations of the author. According to MEZ the four central and empty cells of the hair are free from air and collapsed when dry; but when the thickened portion of the shield absorbs water the appressed walls are forced apart, leaving lumina into which water passes because of negative pressure. The author finds that negative pressure is not a factor at all, and bases this conclusion upon a study of the mechanics of cohesion involved in the shrinkage of artificial cells to which he finds natural cells are comparable. The author first demonstrates that water exercises a cohesive power, which being so well known is perhaps unnecessary. Next he shows that the shrinkage and collapse of artificial cells occurs in a vacuum as well as under ordinary pressure; also that the tension present in a membrane through which water is passing to supply evaporation is independent of air pressure. In the latter case water placed on the surface of such a transpiring membrane is quickly drawn inside because the cohesion pull of the water already inside extends through the fine pores of the membrane. Of course the greater the elasticity of the membrane the stronger cohesion pull it will support and the greater its capacity for bringing outside water within the cell. It is in this relation that the thickened *Deckel* of the scale plays a rôle and not as MEZ found.—RAYMOND H. POND.

KRASNOSSELSKY [10] has made a study of the influence of injury on the activity of the respiratory enzyme in the onion. In agreement with numerous other investigators he finds that injury does increase the respiratory activity of vegetable tissues, and points out that STOKLASA's failure to confirm this observation was due to his not allowing his experiments to run for a sufficient length of time, and that his belief that the results of other workers were due to bacterial con-

[9] STEINBRINCK, C., Einführende Versuche zur Cohäsionsmechanik von Pflanzenzellen nebst Bemerkungen über den Saugmechanismus der wasserabsorbierenden Haare von Bromeliaceen. Flora **94**: 464–477. 1905.

[10] KRASNOSSELSKY, T., Bildung der Amungsenzyme in verletzten Pflanzen. Ber. Deutsch. Bot. Gesells. **23**: 142–155. 1905.

tamination is unfounded. Respiration increases gradually after injury, and it is only after several days that the maximum activity is reached. From that time the process goes on more slowly and finally returns to the normal. By grinding the onions with sand and expressing the juice with a Buchner press, he obtains solutions which liberate carbon dioxide, apparently through the agency of an enzyme. After injury this respiratory enzyme shows an increase in its activity, an increase which reaches a maximum somewhat later than the maximum respiration of the tissues from which the extracts are obtained. Onions whose cells are killed by freezing yield more active enzyme solutions than those not previously frozen. These expressed juices give the oxidase reaction with guaiacum, the juices from injured tissues more vigorously than those from uninjured ones.—ARTHUR L. DEAN.

VINES[11] has given the results of a number of experiments carried out for the purpose of throwing light on the nature of the tryptic enzymes of plants. He assumes that if the powers of a plant extract to convert native proteid into proteases and peptones on the one hand, and to reduce protones to the final cleavage products on the other, do not vary concomitantly under the influence of outside influences, then the two processes are carried out by separate enzymes. Experiments were conducted with the enzymes of *Carica Papaya*, *Ananas sativus*, *Saccharomyces Cerevisiae*, *Agaricus campestris*, *Hordeum sativum*, *Hyacinthus orientalis*, and *Nepenthes*. The proteids used were blood fibrin as a native proteid, and Witte peptone as a proteose and peptone mixture. The factors used to produce variation in proteolytic activity were changes in reaction. In every case it was found that solution of fibrin and cleavage of Witte peptone were affected differently by changes in reaction. VINES concludes that the two processes are carried out by different enzymes; the first stage by enzymes of the pepsin type; the second by those of the erepsin group. He is of the opinion, therefore, that pepsin-like enzymes do occur in plants and that the tryptic action is due to the combined action of such enzymes and those of the erepsin group.—ARTHUR L. DEAN.

MASSART'S[12] interesting experiments with geophilous plants should have been noted long since. In the case of the subterranean stock he sees a conflict between the depth of its burial and the development of aerial shoots. In each plant, therefore, there is a most favorable depth of the subterranean stock which is secured and maintained. Experiments were performed involving about two hundred species of plants, well distributed throughout monocotyledons and dicotyledons. Each species was treated in three lots: one lot very near the surface; another 10^{cm} deep; the third 20 to 30^{cm} deep. The results are outlined very briefly under two heads: methods of ascending when planted below the normal depth; and methods of descending when above the normal depth. The

[11] VINES, S. H., The proteases of plants. III. Ann. Botany **19**: 171–188. 1905.

[12] MASSART, JEAN, Comment les plantes vivaces maintiennent leur niveau souterrain. Bull. Soc. Roy. Bot. Belgique **41**[2]: 67–79. *figs. 12.* 1903.

methods of ascending from too great a depth are stated in outline as (1) elongation of internodes, (2) elongation of internodes and position of buds; (3) localization of buds, (4) curving of the rootstock, and (5) curving of the winter shoots. The methods of descending to a greater depth are (1) localization of the buds, (2) curvature of the rootstock, (3) curvature of the winter shoots, and (4) contraction of the roots.—J. M. C.

JOHNSON[13] has published a preliminary note in reference to his study of the Piperales. In addition to Peperomia, Piper, Heckeria, and Saururus, previously studied, he has studied recently Anemiopsis and Houttuynia (Saururaceae), and also representative genera of Chloranthaceae and Lacistemaceae. The general result is a confirmation of the view that the development of the megasporangium and female gametophyte of angiosperms is not a satisfactory index of genetic relationship, for it may vary widely within a single family or genus. In the genera of Piperales studied there is a variety in the development of the tapetum, megaspore, embryo sac, and endosperm nearly as great as can be found in the whole range of angiosperms. The development of the seed, however, suggests relationships of Piperaceae and Saururaceae to other dicotyledonous families; and the author concludes from such evidence that the Piperales are not very primitive angiosperms, and that they are probably most nearly allied to the four dicotyledonous orders with perisperm-containing seeds—Aristolochiales, Polygonales, Centrospermales, and Ranales.—J. M. C.

MISS RIDDLE[14] has investigated *Batrachium longirostris*, more often regarded as one of the white-flowered species of Ranunculus. So many of the Ranunculaceae have been studied from this point of view that the essential features of the family seem to be well in hand, and in no important respect does the species investigated by Miss RIDDLE change the situation. In the development of the microsporangium there is probably the interesting combination of parietal and sporogenous tissue to form the tapetal layer. It is noteworthy, also, that the male cells, or at least their nuclei, appear just before pollination. In the development of the megasporangium two or more archesporial cells often appear, and no parietal cell is cut off. The antipodals have the character that belongs to the family, retaining the primitive number, but increasing much in size. In the development of the embryo the suspensor is short and somewhat massive, the longitudinal division of the end cell of the proembryo occurring when it consists of three cells.—J. M. C.

SABLON[15] has studied the development of the sporogonium of mosses with the view of comparing it with the development of the stems of vascular plants,

[13] JOHNSON, DUNCAN, S., Seed development in the Piperales and its bearing on the relationship of the order. Johns Hopkins Univ. Circ. No. 178. pp. 28–31. 1905.

[14] RIDDLE, LUMINA C., Development of the embryo sac and embryo of *Batrachium longirostris*. Ohio Nat. **5**: 353–363. *pls. 22–24.* 1905.

[15] SABLON, LECLERC DU, Sur le développement du sporogone des mousses. Rev. Gén. Bot. **17**: 193–197. *figs. 3.* 1905.

carrying forward a point of view suggested in 1878 by KIENITZ-GERLOFF. The sporogonium described is that of *Funaria hygrometrica*, although *Bryum nutans* was also studied. SABLON states that the first periclinal division of the apical segments differentiates a cortical-epidermal region from a central cylinder. The former region continues centrifugal periclinal divisions until the last or so-called epidermal layer is differentiated. This late differentiation of the outermost layer is a feature of the pteridophytes and not of seed-plants. The innermost or oldest layer corresponds to the endodermis of vascular plants. The central cylinder, on the other hand, shows a centripetal succession in its periclinal divisions, the outermost layer, giving rise to sporogenous tissue, being the oldest and corresponding to the pericycle of vascular plants.—J. M. C.

NEWCOMBE[16] has applied three methods to the determination of the angle for maximum response of primary roots and stems. The method of noting the perception time did not give decisive results, although a shorter perception time for a deviation of 90° was indicated than for 135°. The method of noting the after effect did not yield satisfactory results. The method of alternate stimulation at 90° and at 135° deviation from position of stable equilibrium gave very positive results in favor of the former angle. These results discredit the conclusion of CZAPEK that the strongest stimulation occurs at a deviation of 135°. The author's conclusion, recently announced, that orthotropic roots and stems do not receive equal stimulation at equal angles above and below the horizontal, is withdrawn, and support is given to FITTING's view that equal stimuli are received at equal angles above and below the horizontal.—RAYMOND H. POND.

FIGDOR[17] finds that the sheathing leaf base of grasses, in addition to protecting and supporting the unfolding bud, performs the function of a guiding organ. While the growing apex of the young shoot is still enclosed by the cotyledon, the latter, being sensitive to light and gravitation, assumes a favorable position into which the emerging leaf is directed. Coincident with the protrusion of the leaf the growth of the cotyledon ceases and its sensitiveness to light and gravitation disappears. This guiding function of the cotyledon is then assumed by the sheathing leaf base, as the author finds, by virtue of its sensitiveness to light and gravitation. The blade is not sensitive to light, but the vaginal portions of the sheath are and in such portions the sensibility is uniform. The evidence for regarding the sheath as sensitive to gravitation might be more convincing.—RAYMOND H. POND.

HIGHLY SPECIALIZED plant cells and their peculiarities are discussed by DAVIS[18] in a continuation of his studies upon the plant cell. The forms con-

[16] NEWCOMBE, F. C., Geotropic response at various angles of inclination. Ann. Botany **19**:311–323. 1905.

[17] FIGDOR, W., Ueber Heliotropismus und Geotropismus der Gramineenblätter. Ber. Deutsch. Gesells. **23**:182–191. 1905.

[18] DAVIS, B. M., Studies on the plant cell. III. Section 3. Highly specialized plant cells and their peculiarities. Amer. Naturalist **38**:571–594, 725–760. 1904.

sidered are the zoospore, sperm, egg, spore mother cell, coenocyte, and coenogamete. Sperms and eggs are compared with the zoospores with which they are phylogenetically related. After considering the literature of the blepharoplast, the writer is inclined to the view that it does not represent a centrosome. The statement that the synergid may possibly represent portions of a reduced archegonium is somewhat surprising. The author believes that there is no qualitative reduction during the mitoses in the spore mother cell. Pallavicinia receives particular attention. About one hundred and twenty papers are cited in the bibliography of this section.—C. J. CHAMBERLAIN.

SHREVE[19] has investigated the morphology of *Sarracenia purpurea*. The microsporangium passes the winter in the mother cell stage, a two-layered tapetum is developed, the reduced number of chromosomes is twelve, and the tube and generative nuclei appear before the shedding of the pollen. In the megasporangium the integument is single, no parietal cell is cut off, and a linear series of four spores usually appears, although there are variations in number and arrangement. The functional megaspore (innermost one) destroys the overlying nucellar layer at the micropylar end and comes to lie directly against the integument. The endosperm has developed extensively when the embryo is two-celled. In germination the cotyledons act as haustoria, "and survive as simple liguliform leaves bearing chlorophyll."—J. M. C.

FRITSCH[20] claims that the cells of the Cyanophyceae are provided with a delicate cell immediately investing the protoplast in addition to the sheath, which is characteristic of many forms or of mucilaginous envelops. The inner investment is regarded as a modified plasma membrane of a viscous gelatinous nature. The outer envelop is called the cell-sheath, and is believed to be a modified innermost layer of the external mucilaginous investment. This view is quite different from that of most algologists, who regard the sheath as directly derived from the protoplast. FRITSCH also believes that the intercellular protoplasmic connections described by other authors are due to peculiarities in the staining of the gelatinous partitions between the cells.—B. M. DAVIS.

THE LAMINARIACEAE pass through several phases in their life histories, which have been grouped as the embryonal and the post-embryonal. The embryonal stages include the periods up to the time when the simple laminarioid frond is developed; and the post-embryonal the later changes leading to the adult condition which is so various in the different genera. Considerable attention is likely to be given to the post-embryonal stages of development, which promise to throw much light on the problems of relationship. YENDO's work in 1902–3 on Echlonia, Eisenia, and Hedophyllum has recently been supplemented by an

[19] SHREVE, FORREST, The development of *Sarracenia purpurea* L. Johns Hopkins Univ Circ. No. 178. pp. 31–34. 1905.

[20] FRITSCH, K., Studies on the Cyanophyceae. II. Structure of the investment and spore-development in some Cyanophyceae. Beih. Bot. Centralbl. **18**: 194–214. *pl. 1.* 1905.

investigation of SETCHELL[21] on the last two genera and Thalassiophyllum.—B. M. DAVIS.

GWYNNE-VAUGHAN[22] has had the opportunity to study the anatomy of the Chinese marattiaceous genus Archangiopteris, established in 1899 by CHRIST and GIESENHAGEN. Only a single small specimen was available, but if it represents the structure of the larger stems, the genus has a simpler anatomical structure than any of the other Marattiaceae. The single internal vascular strand characteristic of young plants of Angiopteris, Marattia, and Danaea, persists in the mature stem of Archangiopteris. The sporangia were examined by Professor BOWER and reported as corresponding very closely in structure to those of Angiopteris.—J. M. C.

IN AN INVESTIGATION of the fluctuations in the number of ray-flowers of *Chrysanthemum segetum*, LUDWIG[23] has attempted to answer the question how large a number of heads must be counted to insure trustworthy determination of the modes. By counting in lots of fifty heads and adding the results, he comes to the conclusion that in this species 500 heads may be considered the lower limit; that in most species 1000 counts are necessary; and in some 10,000 or even 20,000. He deprecates the work done by American and English investigators who have contented themselves with biometric analysis of a couple hundred observations.—G. H. SHULL.

SETCHELL[24] gives a brief account of several parasitic red algae found on the coast of California and describes a new genus, *Peyssonneliopsis epiphytica* Setchell and Lawson, "growing in small dark red pustules scattered over the surface of membranaceous Rhodophyceae, sending rhizoidal filaments deep into the tissue of the host plant; antheridia and cystocarps unknown." The form is said to differ from Cruoria "only in its parasitic habit and consequent possession of rhizoidal filaments penetrating the host plant." It may perhaps be questioned whether such characters alone justify the establishment of a new genus.—B. M. DAVIS.

WHITE[25] has narrated again for the benefit of the general public his interesting experiences with tomatoes. He describes two separate instances in which seed from the Acme variety of *Lycopersicum esculentum* produced only plants of the potato-leaved tomato, which he calls *L. solanopsis*, and the latter then bred

[21] SETCHELL, W. A., Post-embryonal stages of the Laminariaceae. Univ. Calif Pub. Botany **2**:115–138. *pls. 3.* 1905.

[22] GWYNNE-VAUGHAN, D. T., On the anatomy of *Archangiopteris Henryi* and other Marattiaceae. Ann. Botany **19**:259–271. *pl. 10.* 1905.

[23] LUDWIG, F., Zur Biometrie von *Chrysanthemum segetum*. Festschr. zu Ascherson's 70 stem Geburtstag. pp. 296–301. 1904.

[24] SETCHELL, W. A., Parasitic Florideae of California. Nuova Notarisia. **16**: 59–63. 1905.

[25] WHITE, C. A., The mutations of Lycopersicum. Pop. Sci. Monthly **67**:151–161. *figs. 2.* 1905.

true to its new characters. The author gives repeated assurance that the care taken with these plants leaves no possibility of error. He does not consider the theoretical possibility that his plants were the "extracted recessives" in second-generation Mendelian hybrids instead of mutations.—G. H. SHULL.

STEINER[26] has found intumescences on the leaves of *Ruellia formosa* and *Aphelandra Porteana*, and traced their development. Excessive humidity is found to be the determining condition, as is already known in the case of several other plants. Submersion and darkness each inhibit the appearance of such swellings, while wounding or poisoning cannot be used to induce their formation. The author has evidently overlooked ATKINSON's work of several years ago, in which excessive humidity was found to be important in causing oedema of tomato. —RAYMOND H. POND.

BARBER[27] has given an account of the haustoria of the roots of *Santalum album*. It seems that those in charge of sandal plantations were for a long time uncertain as to the parasitic nature of this tree. There is a certain amount of selection as to hosts, certain plants being much more efficient "nurses" than others. The haustorium arises independently of the presence of any foreign rootlets. When there is contact with such a rootlet the haustorium applies itself closely to its surface, enlarges, and assumes a "conical or bell-like form."—J. M. C.

MISS BERRIDGE[28] has discovered and studied two new specimens of the Carboniferous strobilus described by SCOTT as *Spencerites insignis*, of which only four specimens were known. In consequence, the original diagnosis is considerably modified, but the relationship to other paleozoic Lycopods as outlined by SCOTT remains unaffected.—J. M. C.

THE FISHER FOLK of the Hawaiian Islands apply the term "limu" to the seaweeds of their coasts. They make use of a large number of forms as food and garnishes with fish, shrimps, and limpets. SETCHELL[29] gives a lengthy list of the native names, identifying them in many cases with particular species.—B. M. DAVIS.

LEAVITT and SPALDING[30] have announced their determination of parthenogenesis in *Antennaria fallax* and *A. neodioica*, and the great probability of its occurrence in *A. canadensis* and *A. Parlinii*. A detailed account, with drawings, will be published later.—J. M. C.

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[26] STEINER, R., Ueber Intumeszenzen bei *Ruellia formosa* Andrews und *Aphelandra Porteana* Morel. Ber. Deutsch. Bot. Gesells. **23**:105–112. *pl. 2*. 1905.

[27] BARBER, C. A., The haustoria of sandal roots. Indian Forester **31**:189–201. *pls. 14–19*. 1905.

[28] BERRIDGE, MISS E. M., On two new specimens of *Spencerites insignis*. Ann. Botany **19**:273–279. *pls. 11–12*. 1905.

[29] SETCHELL, W. A., "Limu." Univ. Cali. Pub. Botany **2**:91–113. 1905.

[30] LEAVITT, R. G., and SPALDING, L. J., Parthenogenesis in Antennaria. Rhodora **7**:105. 1905.

NEWS.

PROFESSOR STRASBURGER has been awarded the gold medal of the Linnean Society of London.

DR. W. B. MCCALLUM has been appointed assistant in plant physiology in the University of Chicago.

PROFESSOR CHARLES R. BARNES, after spending six months in Europe, will return to his duties at the University of Chicago October 1.

MR. J. W. RITCHIE, Fellow in botany at the University of Chicago, has been appointed Professor of Biology at William and Mary College, Virginia.

PROFESSOR JOHN M. COULTER and DR. H. C. COWLES will spend the autumn and winter in Europe, returning to their duties at the University of Chicago April 1, 1906.

H. S. GRAVES, School of Forestry, Yale University, has returned from a study of the forest conditions to be observed in a trip around the world, special attention having been given to those of India.

MR. JESSE M. GREENMAN, recently of the Gray Herbarium, Harvard University, has been appointed assistant curator of the Department of Botany at the Field Columbian Museum. His duties began June 1.

A VERY INTERESTING ACCOUNT of a visit to LUTHER BURBANK by Professor HUGO DEVRIES is published in *Popular Science Monthly* for August. It is translated from the Dutch, and contains a clear statement by a great scientific plant-breeder of the scientific results of the work of a great practical plant-breeder.

A NEW BOTANICAL INSTITUTE has recently been completed for Professor RITTER VON WESTERSHEIM R. WETTSTEIN in the Imperial Botanical Garden at Vienna. The building is admirable both for the artistic beauty of its architecture and interior finish, and for the completeness of its equipment. It is well arranged for research and instruction. The museum, so often a mere accumulation of rubbish, is particularly attractive and useful.